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Gi. Melz, Gu. Melz[1], F. Hartmann[2]

[1]PZG Pflanzenzuechtung GmbH, D–1 8276 Gülzow, [2]Kruse Saatzucht GmbH, D–48155 Munster

GENETICS OF A MALE–STERILE RYE OF 'G–TYPE' WITH RESULTS OF THE FIRST F_1 –HYBRIDS

ABSTRACT

Male–sterile plants of 'G–type' were found in rye cv. Schlagler alt. The major recessive gene controlling this male–sterility is allelic with male–sterility genes of the C– and R–types. The genetic system of the Pampa–type is completely different; it is controlled by dominant gene(s) and mt–DNA showed different restriction fragment patterns. The major gene of G–type *msl(Rfgl)* is located on chromosome arm 4RL; the sites of the minor genes *ms2* and *ms3* were found on chromosomes 3R and 6R. Hybrids produced by crossing malesteriles of G–type with inbred lines have normal pollination and, are therefore less sensitive to ergot, like population rye. In 2000 the mslnr–system of G–type rye was registered as –Guelzower1 ". Male–sterile plants of "Guelzowerl" were used to produce the rye hybrid cultivar "Novus", which was also registered in 2000. "Novus" is the first rye hybrid cultivar with resistance to powdery mildew.

Key words rye, male–sterility, inheritance, ergot, powdery mildew, Fl–hybrids

INTRODUCTION

In the early 1960s a program to find male–sterile rye plants was started at the Plant Breeding Institute Gülzow — Gustrow (GDR). The screening of an international collection resulted in 124 male–sterile plants and about 4300 plants were tested as pollinators. Nearly all of these were restorers. Only a few of the crosses resulted in male–steriles and seven male–sterile systems were established. These were either called V–types, or G–types depending on their origin, and consisted of male–sterile lines and the corresponding nonrestorer line (Hahn & Grabow 1975). The main problem of all these systems was incomplete self–fertility in nonrestorer lines (Grabow 2001, pers. corn.).

In '1974 a rye genetics program was initiated at the Plant Breeding Institute Gülzow — Güstrow (GDR). The first character analysed was plant height of the dominant mutant EM–I (Sturm & Engel 1980). Genetic analysis of markers, self–fertility, antinutritive factor, powdery mildew and leaf rust were subsequently investigated (Melz 1988, Melz *et al.* 1990,

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Thiele *et al.* 1989, MeIz and Rollwitz 1991, MeIz and Bielka 1993). Male–sterility has been the main object of genetic research from 1983 to 1987, and we report in this paper the results of that research and the use of malesterility in rye breeding.

MATERIAL

– The following inbred lines, male–sterile lines, trisomics and markers were available at the Department of Genetics, Plant Plant Breeding Institute Guelzow:

Code	Character
Esto	standard tester
IR....	SRtrisomics of Esto (Sturm and Engel 1980)
347&729	inbred line of Petkuser
543S.	silvestre–bastard (Szigat, unpublished)
572	inbred line of Schl~gIer alt
734	Norddeutscher Champagner
975	inbred line of Carstens
ct1	marker compactuml
fertP	male fertile line in P–plasma
msG	male sterile line of G–type (Hahn and Grabow 1975)
nrG	nonrestorer line of G–type
msl	male–sterile found in Imperial rye
msM	male–sterile found in *S. montanum* (Szigat, unpublished)
msP	male–sterile line of P–type (originally described by Geiger and Schnell 1970, adapted by K. Adolf, unpublished)
wal	marker waxless1
(msC)	the male–sterile line of C–type originated from AR Szczecin (Łapiński 1972).
(msR)	the male–sterile line of R–type was donated by VIR St. Petersburg (Kobyljansky and Katerova 1973).

Code	Character
(KM4.... KM3I)	'G–type' hybrids were produced by PZG Pflanzenzuechtung GmbI–I GüIzow. Male–sterile cv. Gülzowerl is resistant to powdery mildew.
DKMIis	registered in E.C. as cv. Novus, and resulted from cooperation between PZG Pflanzenzüchtung GmbH Gülzow (male–sterile cv. Gülzowerl), Danko Hodowla Roslin Sp.z.o.o. (restorer Valet) and Kruse Saatzucht GmbH Muenster (distributor).

METHODS

1. Male–sterile plants of lines msC, msG, msl, msM and msP were crossed with Esto and genetic marker stocks *ctl* and *wal* in order to study the inheritance. Segregation ratios obtained in F_1, F_2 and BC_1 generations were statistically analysed by Chi^2–tests (Weber 1978).
2. Inbred lines and ms–lines were crossed with the C–type nonrestorer (nrC) to investigate the cytoplasmic effect on male–sterility and to obtain information about the identity of genes controlling male–sterility.
3. Trisomics of Esto (I R....6R) were crossed with the C–type nonrestorer (nrC). Male–sterile plants (msG) were pollinated with the resulting F_1 trisomics. Segregating progenies of these back–crosses were analysed for male–sterility to identify the chromo–somes on which the ms–gene is located.
4. Male–sterile cv. Gulzowerl was crossed with inbred lines to produce F_1 hybrids. These hybrids were yield tested at five trial sites (9 m^2/plot, 4 replications).

RESULTS AND DISCUSSION

Inheritance of male–sterility

From the results shown in Table 1 it can be deduced that there are two genetic systems controlling male–sterility in rye. C–, C–, I–, M– and R–type are all controlled by recessive genes because F_1 plants were male–fertile. Allelism tests (Table 2) resulted in sterile F_1 plants from crosses between C– and C–types and R– and C–types. Hence, the same loci control the C–, C– and Rtypes of male–sterility. I– and M–types are controlled by another gene. F_2 segregation ratios (3:1) show that there is no complementary effect of both genes.

Table1

Segregation of crosses among six types including male sterility and the standard tester (Esto) or markers

Combination	F_1	Observed F_2 segregations		χ^2 values of expected segregations			
		fertile	sterile	3 : 1	1 : 3	1 : 7	1 : 15
msC × Esto	fertile	340	112	0.1			
msG × Esto	fertile	145	45	0.2			
msG × ct1	fertile	295	100	0.1			
msG × wa1	fertile	230	60	2.9			
msl × Esto	fertile	90	24	0.9			
msM × Esto	fertile	592	191	0.2			
msR × Esto	fertile	68	21	0.1			
(msP × Esto) × Esto – 1	sterile	10	36		0.1		
(msP × Esto) × Esto 2	sterile	7	69			0.1	
(msP × wa1) × wa1 – 1	sterile	4	139				3.0

Values for significance at 1 DF: 3.84 ($P<0.05$)

Table 2

Segregations of crosses among male–sterile types and G–type nonrestorer line nrG

Combination	F_1	Observed F_2 segregation		χ^2 values of expectedsegregations			
		fertile	sterile	3 : 1	15 : 1	1 : 3	1 : 7
msR × nrG	sterile	–	–				
msC × nrG	sterile	–	–				
msl × nrG	fertile	64	14	2.1			
msM × nrG	fertile	49	17	0.1			
(msP × nrG) × nrG	sterile	16	115				0.1
fertP × nrG –1	fertile	124	17	12.3*	0.1		
fertP × nrG –2	fertile	102	27	1.0			

Values for significance at 1 DF: 3.84 ($P<0.05$)

From thousands of test crosses of male–sterile C–type plants with inbred lines, it was established that male–fertility will be restored without any problems. Nearly all F_1 plants showed full restoration of male–fertility.

In the case of the P–type male–sterility the F_1 hybrids were male–sterile (Table 1) indicating that male–sterility is controlled by dominant genes. Backcrosses with the same partner showed segregations of 1:3, 1:7 and 1:15. These segregations indicate that from one to four dominant genes control inheritance of male–sterility of the

P–type. However, it is possible that more dominant or recessive *ms*–genes are involved in the expression of this male–sterility.

Other research (for instance Ruebenbauer *et al.* 1984) attempted to explain the inheritance of P–type male–sterility by the multiple action of recessive genes. However, male–sterility based on the F_1 generation cannot be used to confirm such a theory. Dominant inheritance was found in cotton (Allison and Fischer 1964) and rape seed (Mathias 1985) and it is also the simplest explanation for the incomplete restoration of male–fertility in hybrids produced from P–type male–steriles and the resulting problems with ergot.

Crosses between P–type male–sterile plants with the C–type nonrestorer nrC also resulted in sterile F_1s. After backcrossing to nrC a 1:7–segregation was obtained (Table 2) and confirmed dominant inheritance.

However, on the basis of this result it was impossible to deduce the relationship between cytoplasmic and nuclear genes of the P– and C–types. Therefore, additional tests were carried out using male–fertile plants (fertP) with P–type cytoplasm. The male–fertile plants used for crosses were the same plants segregating in backcrosses with Esto (Table 1). Crosses with the Ctype nonrestorer (nrC) resulted in male–fertile F_1 plants (Table 2). In the F_2 generation 3:1– and 15:1–segregations were found, indicating the action of one and two recessive ms–genes, respectively. At first it seemed that the malesterility observed was controlled by an ms–gene of C–type nonrestorer (nrC).

However, the identity of these genes is unclear because it is possible that recessive genes also originate from P–type or Esto.

Identification of cytoplasm

Because male–sterile lines and nonrestorers are known in different male–sterile systems it was concluded that cytoplasm inducing male–sterility could be identified by crossing with nonrestorers. The results of crosses between inbred lines and C–type nonrestorer (nrC) are presented in Table 3. In the F_2 generations of crosses of the inbred lines originating from cv. Schlagler alt (572) and cv. Norddeutscher Champagner (734) with nonrestorer nrC a segregation ratio of 3 fertiles: I sterile was found. Hence, both cultivars have cytoplasmic genes interacting with nuclear genes to produce male–sterility.

The F_2 generations of inbred lines originating from other cultivars showed no male–steriles or segregation of 15 fertiles: I sterile. This indicates that these lines have nonrestorer cytoplasm. In the case of inbred line 975 there was in F2 generation 975/1 no male–sterile plant but in F2–generation 97512 (and in combination with line 534) a segregation of 15 fertiles: I sterile. This indicates the presence of a second gene that acts together with the *ms*–gene of C–type. It probably originates from the inbred line where it was not expressed because of the absence

of the second gene. However, there could be also more unknown recessive genes influencing male-sterility.

Table 3

Segregations of crosses among rye genotypes of different origins and G-type nonrestorer line nrG

Combination	F_1	Observed F_2 segregations		χ^2 values of expected segregations	
		fertile	sterile	3 : 1	15 : 1
Esto × nrG	fertile	312	0		
347 × nrG	fertile	93	0		
729 × nrG	fertile	160	0		
975 × nrG – 1	fertile	120	0		
975 × nrG – 2	fertile	115	7	7.5*	0.1
543 × nrG	fertile	64	4	13.2*	0.1
572 × nrG	fertile	55	28	3.4	
734 × nrG	fertile	73	32	1.7	

Values for significance at 1 DF: 3.84 ($P<0.05$)

In order to establish the relationship between nuclear genes of the G- and Ptypes of male-sterility, both types were crossed, too. However, because of the dominant inheritance of P-type, it was impossible to obtain conclusive results. The P- and C-type cytoplasms were investigated using mt-DNA by Steinborn *et al.* 1993; both types showed different mt-DNA patterns.

Chromosomal locaton of ms-genes

The trisomics of Esto were crossed with the C-type nonrestorer nrC. Because the additional chromosomes were not transferred by pollen, male-sterile plants (msC) had to be pollinated by F_1 trisomics. The location of an ms-gene on chromosome 7R was discounted after performing linkage tests. The marker genes *wal* and *ctl* (both located on chromosome 7R, Schlegel *et al.* 1998) showed no linkage with gene *msl*. The resulting backcross generations of trisomics I R — 6R (Table 4) showed unexpected results. The segregation ratios of trisomics 3R, 4R and 6R did not fit the 1:1 – segregation expected for monogenic recessive inheritance. It was concluded, therefore, that there is a major gene *msl* on chromosome 4R and modifying genes are located on chromosomes 3R and SR. Boerner *et al.* (1998) confirmed this result by RFLP-analysis and located gene *msl* (called also *Rfgl)* on chromosome arm 4RL.

The origin of the other two genes *(ms2* and *ms3)* is unknown, but it is possible that they originate from the trisomics. These genes could not be expressed in

trisomics because there was no interaction with the cytoplasm of Esto. It was shown earlier (Table 3) that Esto and msG have different

cytoplasms. Therefore, after backcrossing ms–plants with F_1 trisomics the genes originating from Esto were in an different cytoplasm and could be expressed and even interacted with gene *msl.*

Table 4

Trisomic analysis of male–sterility of the G–type – backcross

Combination	F_1	Observed F_2 segregation		χ^2 values of expectedsegregations			
		fertile	sterile	1 : 1	1 : 2	2 : 1	5 : 1
msG × (1R × nrG)	fertile	64	64	0.0			
msG × (2R × nrG)	fertile	109	100	0.4			
msG × (3R × nrG)	fertile	241	47	130*		0.1	
msG × (4R × nrG)	fertile	89	192	38*	0.4		
msG × (5R × nrG)	fertile	43	41	0.4			
msG × (6R × nrG)	fertile	84	50	6.5*	2.1		

Values for significance at 1 DF: 3.84 ($P<0.05$)

It is completely unknown whether the genes described here and the ms–genes found by Miedaner *et al.* 2000 are allelic.

F_1–hybrids on basis of Gülzowerl (msGI)

At the beginning of the I 990s it became clear that the P–type hybrids have incomplete restoration of male–fertility and this was the reason for ergot epidemics in hybrid rye.

The first hybrids using C–type male–steriles produced in the I 970s showed similar yields as hybrids obtained from P–types (Schmiechen, pers. com.). However, there was the important difference that C–type hybrids had complete restoration of male–fertility in nearly all crosses with other rye genotypes. This was the main reason to start together with Kruse Saaten Spenge a new breeding project to select hybrids on the basis of C–type male–sterility.

Furthermore, resistances to powdery mildew and leaf rust were integrated in the project. The strategy is to produce male–sterile lines with combined resistances to powdery mildew and leaf rust. These resistances must be controlled by dominant genes so that all resulting hybrids will also contain these resistances.

Results from 1997 to 2000 (Table 5) demonstrate that C–type hybrids have the similar yield potential as P–type hybrids. The best results occurred in 1998, when much powdery mildew occurred in early spring. Therefore, in 1998 powdery mildew resistance of all (D)KM — hybrids (Table 5) resulted in high yield, and DKMI (now registered as Novus) was the best hybrid rye in official tests of that year. In 1999 and 2000 the powdery mildew resistance of Novus was not necessary because of the absence of this disease. In these years susceptible cultivars showed

better yield because there was no 'energy penalty' associated with protection against powdery mildew.

Table 5

Results of G–type hybrids in yield trials (9 m² plot, 4 replications, 5 locations)

Hybrid	Year	Relative yield at location					Average
		Chorin	Muckum	Berge	Ebstorf	Gülzow	
DKM1	1997	–	114	–	109	–	111
	1998	–	123	95	105	106	108
KM4	1997	96	103	130	106	115	114
KM5	1997	105	102	101	98	98	101
	1998	–	126	130	101	106	116
KM6	1997	95	119	120	106	110	114
KM9	1998	100	108	107	105	106	107
KM19	1999	–	80	84	86	80	82
KM30	2000	104	105	101	94	97	100
KM31	2000	108	99	96	97	97	99

Low infection with ergot in rye production was observed in years 1999 and 2000. However, in infection tests (Engelke *et al.* 2000, Table 6) it was found that C–type hybrids had much lower numbers of infected spikes than other hybrids. Thus, the real danger of ergot epidemic is still connected with P–type hybrids.

Table 6

Susceptibility of hybrid rye to ergot in 2000 (Engelke *et al.* 2000)

Name of rye	Infected spikes in %	Hybrid type
Halo	7.0	Control (population)
DKM3	3.7	G–type
Festus (DKM5)	6.8	G–type
Novus (DKM1)	10.1	G–type
Avanti	20.3	P–type
Rapid	22.2	P–type

In practical crop production seeds of rye populations are mixed with hybrid rye as a protection against ergot. This system is only practical when there is synchronous flowering of both partners. What will happen when late–emerging sterile spikes remain unpollinated? C–type hybrids have active protection against ergot in this scenario because each plant is fully sterile

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