Theor Appl Genet (1991) 82:761–764

THEORETICAL AND APPLIED GENETICS

Genetic analysis of rye (*Secale cereale* L.) Genetics of male sterility of the G-type

G. Melz and K. Adolf

Institute of Plant Breeding, O-2601 Gülzow-Güstrow, Federal Republic of Germany

Received May 17, 1991; Accepted June 21, 1991
Communicated by R. Hagemann

Summary. The genetics and relationships between the genes in rye located in the nucleus and cytoplasm of the male sterility of the G-type were investigated. A factor inducing male sterility was found in the cytoplasms or rye cv Schlägler alt and rye cv Norddeutscher Champagner. Monogenic inheritance was observed in linkage tests. Using primary trisomics of rye cv Esto, the nuclear gene *ms1* was found to be located on chromosome 4R. Modifying genes, probably masked in normal cytoplasm but expressed in male-sterility-inducing cytoplasm together with gene *ms1*, were located on chromosomes 3R (*ms2*) and 6R (*ms3*). Mono-, di-, and trigenic inheritance types were found in backcross progenies of trisomics.

Key words: Rye – Male sterility – Genetics – Gene location – Trisomics

Introduction

Obermayer (1916) was the first to observe male-sterile rye plants, and Putt (1954) discovered male sterility controlled by the interaction of genes located in the nucleus and the cytoplasm. Genetic analyses of various male-sterile lines found since have produced very different results. Thus Kobyljanskij (1969), Madej (1976), Lapinski (1972), and Gabara et al. (1986) found monogenic recessive inheritance whereas, for instance, Kobyljanskij and Katerova (1973), Scoles (1980), and Ruebenbauer et al. (1984) observed up to five genes controlling male sterility in rye. At present, the male sterility of the P-type selected from 'Pampa' rye by Geiger and Schnell (1970) is that commonly used in breeding. However, inheritance of this male sterility type is very complicated (Ruebenbauer et al. 1984).

Hence, new sources of male sterility showing simple inheritance have been sought. Adolf and Winkel (1985) succeeded in developing such a new type an named it G-type. The male-sterile line of this G-type was selected from rye cv Schlägler alt, whereas the nonrestorer line was found by testcrossing male-sterile plants with inbred lines of different origins.

Studies were made to analyzing the genetics of male sterility and the interactions between genes in the cytoplasm and the nucleus. This paper presents some results of these investigations.

Materials and methods

1. Male-sterile plants of the G-type (msG) were crossed with the marker lines ct1, ct2, An5, ha1, and wa1 in order to analyze inheritance and to find linkages between the gene(s) controlling male sterility (*ms* gene) and the marker genes representing chromosomes 5R and 7R (De Vries and Sybenga 1984; Melz et al. 1988). Linkage was tested using the method described by Weber (1978). Male-steriles were all plants showing either degenerated anther of no pollen in reduced anthers. Fertiles had to be subdivided into semisteriles showing only little pollen in reduced anthers, and fertiles with pollen in normal authors.

2. Inbred lines of very different origin ('Carsten,' 'Esto,' no. 1006, 'Norddeutscher Champagner,' (Petkuser,' 'Schlägler alt,' and *Secale silvestre* bastard) were crossed with the nonrestorer line of the G-type (nonrG) to investigate the influence of the individual cytoplasms on male sterility.

3. The trisomics of rye cv Esto (Sturm 1978; Melz et al. 1988) were crossed with the nonrestorer line of the G-type in order to locate gene *ms1* on chromosomes. The resulting male-fertile F_1-trisomics had to be used as pollinators for male-sterile plants, because it was known from previous analyses that the *ms* gene of the G-type is not expressed in the cytoplasm of rye cv Esto. The resulting progenies were grown in the field and investigated for male sterility. The segregations obtained were tested by Chi-square tests (Weber 1978) for goodness of fit with the expected segregations (Table 1). Trisomic 7R was not available

Table 1. Genetic basis of trisomic analysis – expected segregations in backcross progenies

(a) Monogenic inheritance

Initial cross	Backcross	Expected segregations
AAA × aa	aa × AAa	2Aa:1aa
aaa × AA	aa × Aaa	1Aa:2aa
AA × aa	aa × Aa	1Aa:1aa
aa × AA	aa × Aa	1Aa:1aa
Phenotype		fert. ster.

(b) Digenic inheritance

Initial cross	Backcross	Expected segregations				
		AaBb	Aabb	aaBb	aabb	
AAABB × aabb	AAaBb × aabb	2	2	1	1	2:4
AABBB × aabb	AaBBb × aabb	2	1	2	1	3:3
aaabb × AABB	AaaBb × aabb	1	1	2	2	4:2
aabbb × AABB	AaBbb × aabb	1	2	1	2	3:3
aabb × AABB	AaBb × aabb	1	1	1	1	2:2
AABB × aabb	AaBb × aabb	1	1	1	1	2:2
Phenotype		fert.	fert.	ster.	ster.	

(c) Trigenic inheritance

Backcross	Expected segregations								
	AaBbCc	AabbCc	AaBbcc	aaBbCc	Aabbcc	aaBbcc	aabbCc	aabbcc	
AAaBbCc × aabbcc	2	2	2	1	2	1	1	1	4:2:6
AaBBbCc × aabbcc	2	1	2	2	1	2	1	1	6:1:5
AaBbCCc × aabbcc	2	2	1	2	1	1	2	1	6:1:5
AaaBbCc × aabbcc	1	1	1	2	1	2	2	2	8:1:3
AaBbbCc × aabbcc	1	2	1	1	2	2	1	2	6:2:4
AaBbCcc × aabbcc	1	1	2	1	2	2	1	2	6:2:4
AaBbCc × aabbcc	1	1	1	1	1	1	1	1	4:1:3
Phenotype	fert.	fert.	fert.	ster.	semi.	ster.	ster.	ster.	

because of poor vitality and fertility. However, the location on this chromosome was investigated using the linkage test.

Results and discussion

1. F_1 generations of combinations between male-sterile plants and markers analyzed showed male-fertile plants only. Monogenic 3:1 segregations could be observed in all F_2 progenies (Table 2). It could thus be concluded that the male sterility of the G-type is controlled by one recessive gene. This gene should be named *ms1*.

No linkage between gene *ms1* and the marker genes tested was observed (Table 2). Consequently, it seems feasible to exclude the location of gene *ms1* on chromosomes 5R and 7R revealed by the marker genes used for linkage analysis.

2. Because *ms*-plants and the nonrestorer of the G-type were found in cultivars and inbred lines of different origin, it was concluded that there must be differences in the cytoplasm of male sterility between rye cultivars. Thus, inbred lines of different origin were crossed with the nonrestorer of the G-type to confirm this hypothesis. The resulting F_1 generations were male fertile. However, not only F_2 progenies showed male-fertile plants but also segregating F_2 populations (Table 3). It seems normal that male-sterile plants occurred in the F_2 progeny of cv Schlägler alt, because it was the origin of the G-type and consequently of the cytoplasm, too. However, there was also a 3:1 segregation in the F_2 progeny of cv Norddeutscher Champagner. Thus, both cultivars seem to be of the same cytoplasmic origin or the same cytoplasmic mutations.

Table 2. Results of linkage analyses

Genes tested	Total plants examined	Observed F_2 segregations				χ^2 values		
		A.Ms1	A.ms1ms1	aaMs1.	aams1ms1	3:1	3:1	Linkage
ms1 × An5	484	266	99	90	29	0.1	0.5	0.4
ms1 × ct1	402	235	67	79	21	0.0	2.4	0.1
ms1 × ct2	854	462	177	160	55	0.1	2.1	0.4
ms1 × ha1	236	131	47	42	16	0.1	0.4	0.1
ms1 × wa1	258	149	56	40	13	2.7	0.4	0.2

Values for significance at 1 *df*: 3.84 ($P=0.05$)

Table 3. F_2 segregations of crosses between inbred lines and the nonrestorer of the G-type (nonrG)

Combination	Observed F_2 segregation		χ^2 values	
	fertile	sterile	3:1	15:1
Petkuser × nonrG	93	0		
	160	0		
Carsten × nonrG	120	0		
	113	7	7.5*	0.1
No. 1006 × nonrG	108	0		
S. silvestre bastard × nonrG	64	4	13.2*	0.1
Schläger × nonrG	55	28	3.4	
Norddeutscher Champagner × nonrG	73	32	1.7	
Esto (trisomics) × nonrG	312	0		

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

No male-sterile plants were observed in the F_2 progenies of 'Carsten,' 'Esto', no. 1006, or 'Petkuser.' Consequently, it is impossible to use the trisomics of 'Esto' to locate gene *ms1* in the known way because there would be no expression of gene *ms1* in the F_2 populations. On the other hand, there is no transmission of the additional chromosomes of the trisomics via pollen. Hence, in the first step it was necessary to cross the trisomics with the nonrestorer and, in the second step, male-sterile plants were crossed with the F_1 trisomics. This back-cross method seemed to be the best way to avoid the problems resulting from the combination of unsuitable cytoplasm of 'Esto' and the lack of transmission of addition chromosomes of the trisomics via pollen. However, there were new problems, which are described below.

A small number of male-sterile plants was found in another progeny of 'Carsten' and in the F_2 generation of the cross with the *S. silvestre* bastard. These male-sterile plants probably resulted from a second gene interacting with gene *ms1*. This second gene is obviously expressed only if there is another *ms*-gene. Since both recessive genes were combined, male sterility occurred also in the cytoplasm normally inducing male fertility.

The origin of the cytoplasmic differences between the inbred lines of normal rye is still unknown, but it is very probable that mutations in the mtDNA similar to the mutation found by Tudzynski et al. (1986) in male-sterile 'Pampa rye' are the reason for the effects of different cytoplasms found here. Addition analyses of mtDNA were made to determine the reason for cytoplasmic variability observed and to test the mtDNA constitution of 'Pampa' and G-type.

However, it can already now be concluded from the cytoplasmic differences found that more male sterility systems could exist. The occurrence of these systems inducing male sterility should depend only on other mutations in the cytoplasm and the corresponding genes in the nucleus. Additionally, it is possible that there are many masked genes in common rye. These genes could possibly affect the known male sterility systems if they are crossed into suitable cytoplasm.

On the basis of known cytoplasmic differences and inheritance, it is possible to produce new nonrestorers of the G-type and to identify unknown *ms*-sources. We were able to find nonrestorer plants using testcrosses with male-steriles in various F_2 generations from crosses between no. 1006 and the nonrestorer of the G-type and also in the reciprocal crosses. Additionally, from one male-sterile plant both male-steriles and nonrestorers were constructed by complicated crosses using knowledge of cytoplasmic differences and inheritance.

3. As mentioned, it was necessary to backcross male-steriles with F_1 trisomics to obtain information about the gene location of gene *ms1*. Analysis of backcross progenies was carried out in the summer of 1990. Not only were the clear segregations previously found in linkage analyses observed, but also segregations with three classes (fertiles, steriles, and semisteriles). The number of fertiles and semisteriles was summarized in one class to test monogenic inheritance. It was expected normally that one trisomic with segregation different from disomic 1:1 segregation would be found. However, not only was the expected 1:2 segregation observed in progenies of trisomic 4R, but also segregations different from 1:1 segregation in progenies of trisomics 3R and 6R (Table 4). Because three chromosomes seemed to have influence on trisomic behavior, digenic and trigenic models of inheritance were constructed (Table 1) and tested

Table 4. Trisomic analysis of male sterility of the G-type (backcross)

Combination	Observed segregations			χ^2 values of expected segregations						
	sterile	semifertile	fertile	1:1	1:2	2:1	5:1	4:1:3	4:2:6	6:1:5
msG × (1R × nonrG)	64	0	64	0.0						
msG × (2R × nonrG)	109	5	95	0.4						
	57	15	46	0.1				0.2		
msG × (3R × nonrG)	241	2	45	130.7*			0.1			
msG × (4R × nonrG)	45	16	84	20.9*	0.3			26.5*	2.9	
	44	21	81	23.0*	0.8			24.9*	1.1	
msG × (5R × nonrG)	39	4	41	0.4						
msG × (6R × nonrG)	107	12	100	0.1				12.4*		3.0
	84	4	50	6.5*		2.1				

* Values for significance at 1 *df*: 3.84 ($P=0.05$), at 2 *df*: 5.99 ($P=0.05$)

for goodness of fit. It was found that the segregation observed in progeny of trisomic 2R was not 1:1, but actually trigenic 4:1:3 segregation. The segregations observed in progenies of trisomic 4R were also of trigenic origin. The segregations of trisomic 6R were found to be controlled by either two or three genes. However, it was not possible to interpret the segregation of trisomic 3R. Because the same segregation was found in a trisomic analysis already concluded in 1982, it is very probable that the third gene controlling male sterility of the G-type is located on this chromosome.

The reason for the occurrence of di- and trigenic inheritance is not clear. However, there are some possible interpretations of the results observed.

- There were masked male sterility inducing genes in the trisomics used. These genes were expressed together with the *ms1* gene in the cytoplasm of G-type. Obviously there was one heterozygous gene on chromosome 6R.
- Male sterility of the G-type is controlled originally by more than one gene. However, trigenic inheritance is masked by apparent monogenic inheritance.

Previous investigations showed similar results and trisomic analysis was repeated twice. Although there were always conflicting results, it seems possible to conclude that the major gene controlling male sterility of the G-type is gene *ms1* located on chromosome 4R. The modifying genes on chromosome 3R and 6R should be named *ms2* and *ms3*, respectively.

It is not known whether or not there are more genes affecting the expression of the male sterility of the G-type. However, simple monogenic recessive inheritance was observed in most cases, and male sterility was always restored by any inbred line. The *ms*-lines and nonrestorers must be grown in strict isolation because pollination by normal rye destroys the recessive male sterility system. Although this new type of male sterility is laborious to isolate, it has the advantage of simple inheritance and restoration of male fertility. Hence, male sterility of the G-type could be an alternative to the commonly used 'Pampa' type in hybrid breeding of rye.

References

Adolf K, Winkel A (1985) A new source of spontaneous sterility in winter rye. Preliminary results. Proc Eucarpia Meeting of the Cereal Section on Rye, Svalöv Sweden, pp 293–307

De Vries JN, Sybenga J (1984) Chromosomal location of 17 monogenetically inherited morphological markers in rye (*Secale cereale* L.) using the translocation tester set. Z Pflanzenzücht 91:117–139

Gabara B, Kubicki B, Kubicka H (1986) Study on male sterility in rye (*Secale cereale* L.). Genet Pol 27:1–11

Geiger HH, Schnell FW (1970) Cytoplasmic male sterility in rye (*Secale cereale* L.). Crop Sci 10:590–593

Kobyljanskij VD (1969) Citoplasmaticeskaja muzskaja sterilnosti u diploidnoij rzi. Vestn Skh Nauki 14:18–22

Kobyljanskij VD; Katerova AG (1973) Nasledovanije citoplasmalticeskoj i jaderno-muzskoj sterilnosti u osimoj diploidnij rzi. Genetika 9(7):5–11

Lapinski M (1972) Cytoplasmic-genic type of male sterility in *Secale montanum* Guss. Wheat Inf Serv 35:25–28

Madej L (1975) Research on male sterility in rye. Hodowla Rosl Aklim Nasienn 19:421–422

Melz G, Schlegel R, Sybenga J (1988) The identification of the chromosomes in the 'Esto' set of rye trisomics. Plant Breed 100:169–172

Obermayer (1916) cit. Roemer T (1939) Roggen (*Secale cereale* L.) In: Handbuch der Pflanzenzüchtung, vol 2. pp 1–23

Putt ED (1954) Cytogenetic studies of sterility in rye. Can J Agric Res 34:81–118

Ruebenbauer T, Kubara-Szpunar L, Pajak 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 25:1–17

Scoles GJ (1980) Studies with male-sterile rye. Diss Abstr Int B 40:2979–2980

Sturm W (1978) Identifizierung von Trisomen der Sorte ‚Esto' und Trisomenanalyse des Genes *Hl* für Kurzstrohigkeit bei *Secale cereale* L. Diss Abstr Akad Landwirtschaftswiss DDR, 180 pp

Tudzynski P, Rogmann P, Geiger HH (1986) Molecular analysis of mitochondrial DNA from rye (*Secale cereale* L.). Theor Appl Genet 72:695–699

Weber E (1978) Mathematische Grundlagen der Genetik, 2nd edn. Fischer, Jena, 582 pp.