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OCCURRENCE AND GENETIC IDENTITY OF MALE STERILITY–INDUCING CYTOPLASM IN RYE (*SECALE* SPP.)

ABSTRACT

Individual plants of 50 open–pollinated cultivars originated from 23 world–wide countries, 18 inbred lines and 9 wilde species and/or subspecies of rye were tested for the presence of steril–ity–inducing vs. normal cytoplasm, using conventional plasmotype/genotype interaction test.

One to fourteen random plants from each population were crossed as females to inbred lines representing nonrestorer genotype in both, Pampa and Vavilovii, types of sterility–inducing cyto–plasm. The F_1 and Bc_1 or F_2 progenies were scored for male fertility/sterility expression. The results showed that male sterility–inducing cytoplasm was common in the sample of a world–wide rye population. Out of a total 629 single plants tested, 366 plants had sterility–inducing cytoplasm and all of them were derivatives of a cultivated rye populations. Among 50 cultivars and local popula–tions of cultivated rye either normal or sterilizing cytoplasm was found in 9 and 19 populations, re–spectively, while 22 populations consisted of both plant types, with normal and sterilizing cytoplasm. A random sample of 61 male sterile Bc_2 – Bc_3 single plant progenies (a new sources of *cms*) developed from 28 populations were crossed to the L1 inbred line, acting as nonrestorer in Pampa and as restorer in Vavilovii cytoplasm, in an attempt to identify the type of sterility–induc–ing cytoplasm detected. Fifty of the 61 F_1 progenies were male fertile indicating the presence of Vavilovii cytoplasm. Eleven F_1 progenies were either male sterile or segregating but backcrossing of male sterile segregates resulted in male sterile Bc_1 and Bc_2 progenies, thus indicating that they all had Pampa cytoplasm. These progenies originated from plants of 6 South–American popula–tions.

Key words: cytoplasmic male sterility, genetic resources, plasmotypic diversity, *Secale* spp.

INTRODUCTION

On the basis of what is presently known, cytoplasmic male sterility in rye is characterized by having only two distinct types, the Pampa (*cms–P*) and the Vavilovii (*cms–V*). They are differentiated by genes for plasmon sensitivity or conversely, by genic requirements for fertility restoration (Müller *et al.* 1978; Geiger 1982; Morgenstern, Geiger 1982; Łapiński, Stojałowski 1996). Both types were discovered approximately at the same time (Zdrilko 1969, 1972; Geiger, Schnell 1970; Kobyljanskij

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1971) and both were shown in numerous studies to fulfil the require–ments for successful use in hybrid breeding. Nevertheless, all hybrid cultivars of rye released since 1984 up to 1999 in Germany, and in Po–land as well were developed with use of the cytoplasmic male sterility induced by the Pampa cytoplasm from Argentinian primitive rye. How–ever, in 2000 the rye hybrid cultivar Novus was released in Germany (Bundessortenamt 2000). 'Novus' is the first rye hybrid variety based on the Vavilovii type of sterility–inducing cytoplasm, originated from Ger–man 'Schlägler Alt' population.

Male sterility–inducing cytoplasm have been rediscovered many times in open–pollinated populations, inbred lines, intra–specific and inter–specific crosses of rye in several European countries. Currently, our rough estimate of an amount of a proven sterility–inducing cytoplasms (*cms* sources) approximate 60 or more. Many of them were also tested, more or less precisely, for genetic identity and, with only 4 exceptions, were shown to be different from Pampa and indistinquishable from Vavilovii cytoplasm on the basis of their fertility restoration requirements (Geiger 1971, 1982; Guljaeva 1972; Klučko, Belousov 1972; Łapiński 1972, 1975, 1977; Geiger, Morgenstern 1975; Madej 1975, 1976; Ahokas 1980; Adamčuk, Zdril'ko 1982; Kobyljanskij 1982; Kobyljanskij *et al.* 1982, 1994; Morgenstern, Geiger 1982; Adolf, Winkel 1985; Geiger *et al.* 1994; Łapiński, Stojałowski 1996; Warzecha, Salak–Warzecha 1996). Three exceptions, out of 4 mentioned above, were found in extensive studies of Kobyljanskij *et al.* (1994). Fourty two, out of 45 separate sources of sterility–inducing cytoplasm were shown to be identical to Vavilovii, 1 source, from Kazahskaja weedy–rye, ap–peared to be of Pampa type, and 2 sources might be different from either Pampa or Vavilovii cytoplasm but this was not proven conclusively. The 4th exception came from the paper of Warzecha and Salak–Warzecha (1996). The authors reported that they found male sterility–inducing cytoplasm of Pampa type in the Polish open–pollinated cultivar Dańkowskie Złote.

This short rewiew gives convincing evidence of the widespread exis–tence of sterility–inducing cytoplasms in populations of cultivated rye. However, from the point of view of plant breeding both, the theory and practice, this general conclusion even though correct does not seem to be neither exhaustive nor informative. Our objective in this work was to determine the kind of cytoplasm (inducing vs. noninducing male steril–ity) and the type of the detected sterilizing cytoplasm (Pampa vs. non–Pampa), in a wide range of rye populations. The insight into the plasmotypic structure of populations might have practical significance, in that it should facilitate the development of new restorer and nonrestorer lines in hybrid rye breeding.

MATERIALS AND METHODS

Seed samples of wild rye species were obtained as early as 1965–1966 from Max Planck Institute of Plant Breeding, Volksdorf, Germany (*S. montanum* Guss., *S. kuprijanovii* Grossh.), Institute of General Botany, Humboldt University, Berlin (*S. vavilovii* Grossh., *S. silvestre* Host.) and N.I. Vavilov Institute of Plant Industry, Sankt Petersburg (*S. anatolicum* Boiss., *S. kuprijanovii* var. *chaldicum* Fed.). The wild rye species were at first crossed as females and than backcrossed several times to the 'Smolickie' rye cultivar in an attempt to produce alloplasmic forms of the male parent. Later on autoplasmic and alloplasmic populations of 'Smolickie' were crossed as females and backcrossed nine times to the KaH6 long–term inbred line. The resulting 7 Bc_9 hybrids were used in this study as a sources of an allien cytoplasm.

Seeds of the 3 weedy–rye species were kindly provided by Botanical Garden – Center for the Biological Diversity Conservation of the Polish Academy of Sciences, Warsaw while those of 50 cultivated rye population mostly by National Centre for Plant Genetic Resources, Plant Breeding and Acclimatization Institute, Radzików, Poland. Total 23 inbred lines were also used in the study. Most of them (14) were developed by the senior author of this article at Agricultural University of Szczecin. The remaining lines came from different institutions, mainly from dr L. Madej's collection of Plant Breeding and Acclimatization Institute at Radzików. Regardless of their origin, all but one inbred lines used were maintained in our collection by pedigree method of inbreeding for a long time, enough to consider them homozygous and homogeneous. The exceptional line S436N/95 was obtained recently from DANKO Plant Breeding Ltd of Choryń, courtesy of the late A. Szołkowski, MS.

Six long–term inbred lines were essential to the procedures of testing the kind (sterilizing vs. nonsterilizing) and type (Pampa vs. non–Pampa) of cytoplasm present in individual plants. The lines 541, 542–5, 542–9, 544 and 711, originated from our earlier programe of breeding genotypes acting as nonrestorers in *cms*–C source of cytoplasm, maintaine male sterility in a wide range of sterility–inducing cytoplasms including both, Vavilovii and Pampa type sources. For this reason theese lines hereafter are referred to as double nonrestorers. They are known to have nonsterilizing (normal) cytoplasm and were used to differentiate between sterilizing and nonsterilizing cytoplasm. The test for the presence of sterility inducing cytoplasm proceeded as follows. Individual plants or lines were crossed as females with a double nonrestorer inbred line to introduce nonrestorer alleles into genotypes of the F_1 progenies. Four random plants within each of a singla plant or line F_1 progenies were then backcrossed to a double nonrestorer parent or selfed to bring the recessive nonrestorer alleles to homozygosity. The F_1, BC_1 and/or F_2 progenies of each individual plant or line were grown separately and scored for male fertility/sterility expression. Each prog–

eny was assigned to one of the three classes: male–fertile, male–sterile and segregating. The occurrence of male steriles in a given single plant Bc_1 and/or F_2 progeny was a proof that the initial female plant had sterility–inducing cytoplasm. Segregation in F_1 indicated both, a presence of sterility–inducing cytoplasm and heterozygosity in the *Rf locus(i)* of the initial seed parent plant.

The L_1 inbred line of German origin was used to test the type of sterility–inducing cytoplasm detected in Bc_1 progenies. This line was known to be nonrestorer in Pampa and a potent restorer in Vavilovii sources of sterility inducing cytoplasm. First, four random male–sterile plants from segregating BC_1 generation were crossed back to the male reccurrent parent in order to confirm maternal inheritance of the male–sterile trait. Male–sterile BC_2 or BC_3 progenies, hereafter referred to as a new *cms* sources, were then crossed to the L_1 inbred line and the resulting F_1's were scored for male fertility. Complete male fertility of a F_1 implied the presence of Vavilovii type cytoplasm while male sterility was indicative of the presence of Pampa type cytoplasm.

The hybrid progenies were grown usually in a single–row unreplicated plots in field nurseries at Szczecin. Some of the F_1 progenies, however, were raised in pots if the amount of seed produced was insufficient. Row–plots, spaced at 40 cm, were overseeded and thinned, if necessary, to secure about 10 cm plant spacing within rows.

Throughout this work the level of male fertility was assessed by visually scoring the size and dehiscens of the anthers on a 1 to 9 scale according to Geiger and Morgenstern (1975). Plants rated 1, 2 or 3 were classified as male–sterile (MS), 4, 5 or 6 as partially male–fertile (PF), and 7, 8 or 9 as male–fertile (MF). Two to three spikes per plant were examined at flowering.

RESULTS

Cytoplasm of wild and weedy rye species

Male fertile alloplasmic versions of the KaH6 inbred line with the cytoplasm of *Secale anatolicum, S.kuprijanovii, S.kuprijanovii var. chaldicum, S.montanum, S.vavilovii, S.silvetre,* respectively, and partially male fertile form with the cytoplasm of *S.cereale* cv. Smolickie were crossed each to two double nonrestorer inbred lines in an attempt to determine the kind of cytoplasm present in wild rye species (Table 1). The resulting F_1's possessing cytoplasm of wild species were male fertile and were selfed while those having cytoplasm of cv. Smolickie were nearly male sterile and were backcrossed to obtain segregating generations. The F_2 progenies were all male fertile while the BC_1 progenies were male sterile indicating that the sample of the wild rye species tested had normal cytoplasm, noninducing male sterility and that cv. Smolickie had sterility–inducing cytoplasm. This was consistent with the reaction of the KaH6 line genotype to the presence of the alien

cytoplasms described above. The KaH6 line itself has normal cytoplasm (see Table 4) and a genotype acting as nonrestorer in Pampa and as very weak restorer in Vavilovii sterilizing cytoplasm (Łapiński, Stojałowski 1996).

Table 1

Male fertility of progenies from crosses between alloplasmic versions of the KaH6 inbred line and nonrestorer pollinator lines

Source of cytoplasm	Pollinator line	Generation	Number of plants		
			MF	MS	Total
Secale anatolicum Boiss.	542–5	F2	83	0	83
S. anatolicum Boiss.	542–9	F2	126	0	126
S. kuprijanovii var. *chaldicum* Fed.	541–6	F2	106	0	106
S. *kuprijanovii* var. *chaldicum* Fed.	542–9	F2	101	2	103
S. kuprijanovii Grossh.	542–5	F2	105	0	105
S. kuprijanovii Grossh.	542–9	F2	123	0	123
S. montanum Guss.	542–5	F2	88	0	88
S. montanum Guss.	542–9	F2	81	0	81
S. vavilovii Grossh.	542–5	F2	44	0	44
S. vavilovii Grossh.	542–9	F2	122	0	122
S. silvestre Host.	542–5	F2	64	0	64
S. silvestre Host.	542–9	F2	104	0	104
S. cereale L. 'Smolickie'	542–5	Bc1	0	110	110
S. cereale L. 'Smolickie'	542–9	Bc1	1	83	84

Table 2

Male fertility of F_1 and Bc_1 progenies from crosses between individual plants of weedy rye populations as females and the nonrestorer L544 line as male recurrent parent

Population	No of plants	No of F_1 progenies			No. of Bc_1 progenies		
		Male fertile	Male sterile	Segre–gating	Male fertile	Male sterile	Segre–gating
Secale segetale Zhuk.	13	13	0	0	13	0	0
S. ancestrale Zhuk.	12	12	0	0	12	0	0
S. afghanicum Vav.	12	12	0	0	12	0	0

Thirty seven plants of 3 populations of weedy rye species *Secale segetale, S.ancestrale* and *S.afghanicum* were used in the test for the presence of sterilizing vs. nonsterilizing cytoplasm. Twelve to 13 indi–vidual plants randomly taken from each population were crossed as fe–males with the double nonrestorer inbred line 544. Results from the test are shown in Table 2. None of the F_1 and Bc_1 progeny displayed fertile:

sterile segregation indicating that the weedy rye populations tested were free of plants carrying sterility–inducing cytoplasm. Thus both, wild and weedy rye populations appeared to have only normal cyto–plasm.

Cytoplasm of cultivated rye

Fifty open–polinated cultivars and local populations originated from 23 world–wide countries and 18 inbred lines were involved in the test for the presence of sterilizing vs. normal cytoplasm in cultivated rye. Eight to 14 individual plants from each population and 2 spikes from each in–bred line were emasculated and then pollinated with pollen of the dou–ble nonrestorer line 544. In a few cases, however, the line 711 was used instead of 544, as a pollen parent. Results from the test are shown in Table 3 and 4.

Table 3

Male fertility of F_1 and Bc_1 progenies from crosses between individual plants of cultivated rye populations as females and the nonrestorer L544 line as male recurrent parent

Population	Origin	No of plants	Number of progenies					
			F_1			BC_1		
			Male fertile	Male sterile	Segre–gating	Male fertile	Male sterile	Segre–gating
Abruzzi	ITA	11	10	0	1	9	0	2
Amilo	POL	12	7	0	5	0	0	12
Anatolien	TUR	12	12	0	0	12	0	0
Ancora*	TUR	12	12	0	0	12	0	0
Animo	NLD	12	12	0	0	0	0	12
Antelope	CAN	11	11	0	0	7	0	4
Barroso	PRT	13	11	0	2	2	0	11
Borellus	DEU	14	14	0	0	14	0	0
Candar*	TUR	12	10	0	2	6	0	6
Carokurz	DEU	11	11	0	0	11	0	0
Centeno 52	ESP	12	12	0	0	1	0	11
Cougar	USA	11	11	0	0	9	0	2
Dańkowskie Złote	POL	11	6	1	4	3	1	7
Dominator	DEU	11	10	0	1	0	0	11
Don Enrique	ARG	9	8	0	1	3	0	6
Don Enrique INTA*	ARG	12	0	1	11	0	2	10
Galma	BEL	11	11	0	0	11	0	0
Grand Crouelle	FRA	10	9	0	1	1	0	9
Harlan I.R.6982*	TUR	12	6	0	6	3	2	7
Haru 4	JPN	10	10	0	0	10	0	0
Jarosławna	RUS	12	12	0	0	0	0	12

* The nonrestorer inbred line L711 was used as recurrent parent instead of line L544

Table 3

Male fertility of F_1 and Bc_1 progenies from crosses between individual plants of cultivated rye populations as females and the nonrestorer L544 line as male recurrent parent (continued)

Population	Origin	No of plants	Number of progenies					
			F_1			BC_1		
			Male fertile	Male sterile	Segre–gating	Male fertile	Male sterile	Segre–gating
Kalinka	RUS	10	9	0	1	1	0	9
Kartano	FIN	11	10	0	1	6	0	5
Kenya	KEN	12	12	0	0	11	0	1
Kisvardai	HUN	12	12	0	0	0	0	12
Kungs	SWE	11	8	0	3	0	0	11
Kustro	DEU	10	8	0	2	0	0	10
Madar	POL	12	12	0	0	3	0	9
Maksimirska	YUG	12	10	0	2	0	0	12
Merkator	DEU	12	6	0	6	1	0	11
Mongolskie	MNG	11	11	0	0	11	0	0
Montalegre	PRT	9	9	0	0	9	0	0
Motto	POL	13	8	0	5	0	1	12
Pasteoro Massaux*	ARG	12	8	1	3	5	3	4
Perolo	DEU	8	8	0	0	8	0	0
Pico Gentario*	ARG	12	0	1	11	0	2	10
Pico Mag*	ARG	12	3	1	8	2	3	7
Pico Urugway	URY	12	12	0	0	8	0	4
Pluto	DEU	11	8	0	3	0	0	11
Ponsi	FIN	12	9	0	3	0	0	12
Rheidol	GBR	11	11	0	0	9	0	2
Saratovskaja	RUS	12	11	0	1	0	0	12
Smolickie	POL	9	8	0	1	0	0	9
Tschermaks Marchfelder	AUT	11	9	0	2	0	0	11
Tulunskaja Zelenozernaja	RUS	11	8	0	3	6	0	5
Turkey 75*	TUR	12	12	0	0	11	0	1
Vjatka 2	RUS	11	9	1	1	7	1	3
Warko	POL	13	13	0	0	0	0	13
Wibro	POL	11	11	0	0	0	0	11
Wojcieszyckie	POL	12	6	0	6	0	0	12
Total		568	466	6	96	212	15	341

* The nonrestorer inbred line L711 was used as recurrent parent instead of line L544

The between and within population plasmotypic diversity was apparent. Only 9 (18%) populations were found to be composed solely of plants carrying nonsterility–inducing cytoplasm (Table 3). This group includes primitive land–races and cultivars such as Turkish 'Anatolien' and

Table 4

Male fertility of Bc_1 progenies from crosses between a set of female inbred lines and the nonrestorer line L544 as male recurrent parent

Female inbred lines		Number of Bc1 plants			
Designation*	Source population	MF	PF	MS	Total
620/75–1–5,** S11	Dańkowskie Złote	30	0	0	30
620/75–1–2,** S10	Dańkowskie Złote	29	0	0	29
DS2,** S14	S.dighoricum × 'Smolickie'	25	0	7	32
We109, S16	Wesser	31	0	0	31
Do0–3,** S14	Dominant	17	0	14	31
C632, S14	Sangaste	28	0	0	28
L1, S16	Petkuser Normalstroh	28	0	0	28
L1, S16	Petkuser Normalstroh	289	0	0	289***
L18, S14	Petkuser Normalstroh	18	0	10	28
KaH6, S16	Kazimierskie	28	0	0	28
Ku1–4,** S16	Kungs 2	16	0	12	28
OtO–6,** S16	Otello	55	0	7	62
OtO–20,** S14	Otello	23	0	6	29
OtO–25–1,** S12	Otello	19	4	7	30
Ot1–3,** S15	Otello	17	0	11	28
RoP2, S14	Rogalińskie	30	0	0	30
RoP2, S14	Rogalińskie	281	0	0	281***
RXL10, S14	Zeelandzkie	33	0	0	33
C599/74, S16	unknown	26	0	0	26
S436N/95, S5	MatS × Dominator	0	6	48	54

* The symbol S with a subscript describes number of generations of inbreeding conducted in Szczecin, excluding line S436N/95
** Lines developed in Szczecin
*** F_2 generation

'Ancora', Mongolian 'Mongolskie', Portuguese 'Montalegre' but also older German 'Borellus', 'Carokurz' and Belgian 'Galma' varieties. On the other hand, more than twice larger group of 19 (38%) populations lacked plants with normal cytoplasm; i.e., they were built up of plants with sterility–inducing cytoplasm. An outstanding European cultivars: Polish 'Amilo', 'Motto' 'Warko'; German 'Kustro', 'Dominator', 'Pluto'; Swedish 'Kungs' are representatives of the group. The remaining 22 (44%) populations appeared to contain plants with normal cytoplasm and plants with sterilizing cytoplasm as well. All possible ratios of plants with normal vs. sterilizing cytoplasm within populations were observed; e.g., 1:11 in Spanish 'Centeno 52' and German 'Merkator', 1:1

in Turkish 'Candar', and 11:1 in "Kenya' and 'Turkey 75'. This group in–cludes also Polish cultivars 'Dańkowskie Złote' and 'Madar', most of Rus–sian Turkish and South–American cultivars, North–American and some of Finnish, Italian and Portuguese cultivars tested. In summary, the great majority (41 out of 50, i.e. 82%) of a populations tested carried plants with sterility–inducing cytoplasm either exclusively or in mixture. The presence of a sterility–inducing cytoplasm insofar as tested does not seem to be affected by a geographic origin of a population. Of a total 568 individual plants tested, 356 plants (63%) possessed cytoplasm inducing male sterility to the double maintainer genotype. Therefore it could be safely stated that the sterilizing cytoplasm was of frequent occurrence in cultivated rye populations. On the contrary, normal cytoplasm must be relatively rare.

Rather high level of heterozygosity was detected among plants having sterilizing cytoplasm. Ninety six plants out of 356, i.e. 27% were hetero–zygous at the *Rf locus(i)* as evidenced by segregation for male fertil–ity/sterility trait in F_1. There were also 6 male sterile plants (1.7%) among those tested.

Out of 18 inbred lines tested only 9 appeared to have sterility–inducing cytoplasm (Table 4). These lines were derived from 'Otello', 'Kungs 2' (SWE), 'Petkuser Normal' (DEU), 'Dominant'(NED) *Secale dighoricum* × 'Smolickie' and MatS × 'Dominator' populations. However, a set of lines tested was a selected, rather than random sample of lines. Only line DS2 deserves attention since it most probably has cytoplasm of *S. doghoricum*, another weedy rye species and, on the contrary to *S. segetale, S. ancestrale* and *S. afghanicum* this is sterility–inducing cytoplasm

Type of sterility–inducing cytoplasm

There were a total 365 segregating and male sterile Bc_1 progenies, each being potential source of *cms* (Table 3, 4). Of these, 61 random progenies were advanced to the BC_3 generation by crossing male sterile segregants to the recurrent male parent. Results are given in Table 5. All the Bc_3 progenies obtained were male sterile indicating cytoplasmic inheritance of the trait. Male sterile BC_2 and/or BC_3 progenies, representing a new reliable sources of male sterility–inducing cytoplasm, were crossed to the L_1 inbred line and the F_1 generations were scored for male fertility ex–pression. Results are shown in Table 6. Observation of the resulting test crosses showed that each of the new cytoplasmic sources could be classi–fied as identical in reaction to the Vavilovii or to the Pampa cytoplasm. Fifty of a total 61 F_1 hybrids were male fertile indicating that they carried the cms–V cytoplasm. Fourty seven, out of 50 sources, were derived from European populations, 2 sources were of Canadian origin and one source was developed from Argentinean population 'Don Enrique'. Only three F_1 progenies were male sterile indicating the presence of *cms*–P cytoplasm. Eight progenies apparently segregated for male fertile and male sterile plants; although segregation can be explained easily assuming residual

Table 5

Male fertility of Bc_2 and Bc_3 progenies obtained from crosses of male sterile Bc_1 segregates with the recurrent male parent

Source of male sterile Bc1 plants	Bc_2				Bc_3			
	MF	PF	MS	Total	MF	PF	MS	Total
Abruzzi 201–3 × 544	0	0	50	50	0	1	11	12
Abruzzi 201–5 × 544	0	0	41	41	0	0	33	33
Antelope 204–5 × 544	0	0	36	36	0	0	18	18
Antelope 205–2 × 544	0	0	34	34	0	2	41	43
Barroso 206–3 × 544	0	0	26	26	0	0	26	26
Barroso 206 –5 × 544	0	0	19	19	0	0	27	27
Centeno 52 209 –3 × 544	0	0	25	25	0	0	32	32
Centeno 52 210 –3 × 544	0	0	37	37	0	0	16	16
Dańkowskie Złote 264 –1 × 544	0	0	25	25	0	0	40	40
Dańkowskie Złote 264 –5 × 544	0	0	30	30	0	0	32	32
Dominator 238–1 × 544	0	0	23	23	0	0	14	14
Dominator 238–4 × 544	0	0	50	50	0	0	17	17
Don Enrique 214–1 × 544	0	0	28	28	0	0	17	17
Don Enrique 214–3 × 544	0	0	27	27	0	3	18	18
Don Enrique INTA 320–5 × 711	0	0	42	42	0	0	36	36
Don Enrique INTA 322–1 × 711	0	0	35	35	1	6	30	37
Grand Crouelle 216–1 × 544	0	0	16	16	0	2	12	14
Grand Crouelle 217–3 × 544	0	0	43	43	0	0	32	32
Jaroslavna 251–1 × 544	0	0	35	35	0	0	35	35
Jaroslavna 251–3 × 544	0	0	45	45	0	0	21	21
Kalinka 249–1 × 544	0	0	32	32	0	0	28	28
Kalinka 249–4 × 544	0	0	33	33	0	0	25	25
Kartano 245–2 × 544	0	0	29	29	0	1	37	38
Kartano 246–3 × 544	0	0	28	28	0	0	32	32
Kisvardai 224–1 × 544	0	0	40	40	0	0	45	45
Kisvardai 224–3 × 544	0	0	47	47	0	0	45	45
Kungs 280–2 × 544	0	0	12	12	0	0	32	32
Kungs 280–3 × 544	0	0	9	9	0	2	41	43
Kustro 266–1 × 544	0	0	20	20	0	0	23	23
Kustro 266–3 × 544	0	0	10	10	0	0	40	40

Table 5

Male fertility of Bc_2 and Bc_3 progenies obtained from crosses of male sterile Bc_1 segregates with the recurrent male parent (continued)

Source of male sterile Bc1 plants	BC_2				BC_3			
	MF	PF	MS	Total	MF	PF	MS	Total
L18 × 544	0	0	12	12	0	0	63	63
Madar 236–2 × 544	0	0	27	27	0	0	38	38
Madar 236–3 × 544	0	0	30	30	0	0	17	17
Maksimirska 226–3 × 544	0	0	45	45	0	0	44	44
Maksimirska 226–4 × 544	0	0	28	28	0	1	33	34
Merkator 283–1 × 544	0	0	10	10	0	0	39	39
Merkator 284–2 × 544	0	1	13	14	0	0	19	19
Ot1–3 × 544	0	0	15	15	0	0	62	62
Pasteoro Massaux 324–2 × 711	0	0	41	41	0	0	22	22
Pasteoro Massaux 324–4 × 711	0	0	38	38	0	0	17	17
Pico Gentario 328–3 × 711	0	0	43	43	0	7	22	29
Pico Gentario 330–2 × 711	0	0	42	42	0	0	41	41
Pico Mag 333–3 × 711	0	0	37	37	0	0	39	39
Pico Mag 334–1 × 711	0	1	43	44	0	0	33	33
Pico Urugway 230–2 × 544	0	0	47	47	0	2	31	33
Pico Urugway 230–4 × 544	0	0	24	24	0	0	20	20
Rheidol 233–1 × 544	0	0	39	39	0	0	14	14
Rheidol 233–5 × 544	0	2	40	42	0	0	23	23
Saratovskaja 259–3 × 544	0	0	23	23	0	0	31	31
Saratovskaja 259–6 × 544	0	0	21	21	0	0	19	19
Smolickie 274–1 × 544	0	0	25	25	0	0	37	37
Tschermaks Marchfelder 273–4 × 544	0	0	33	33	0	0	32	32
Tschermaks Marchfelder 273–6 × 544	0	0	22	22	0	0	33	33
Tulunskaja Zelenozernaja 256–2 × 544	0	0	25	25	0	0	24	24
Tulunskaja Zelenozernaja 256–3 × 544	0	0	32	32	0	0	20	20
Vjatka 2 262–1 × 544	0	0	16	16	0	0	31	31
Vjatka 2 262–5 × 544	0	0	21	21	0	0	22	22
Warko 234–2 × 544	0	0	24	24	0	0	49	49
Warko 235–1 × 544	0	0	35	35	0	0	44	44
Wibro 254–1 × 544	0	0	37	37	0	0	43	43
Wibro 254–2 × 544	0	0	46	46	0	0	33	33

Table 6

Male fertility of F_1 hybrids between male sterile Bc_2 and/or Bc_3 progenies and the L1 inbred line

Source of male sterile BC_1 plants	Number of F_1 plants			
	MF	PF	MS	Total
Abruzzi 201–3 × 544	52	0	0	52
Abruzzi 201–5 × 544	72	0	0	72
Antelope 204–5 × 544	45	0	0	45
Antelope 205–2 × 544	44	0	0	44
Barroso 206–3 × 544	30	0	0	30
Barroso 206–5 × 544	54	0	0	54
Centeno 52 209–3 × 544	79	1	0	80
Centeno 52 210–3 × 544	43	0	0	43
Dańkowskie Złote 264–1 × 544	40	0	0	40
Dańkowskie Złote 264–5 × 544	31	0	0	31
Dominator 238–1 × 544	81	0	0	81
Dominator 238–4 × 544	52	0	0	52
Don Enrique 214–1 × 544	72	0	0	72
Don Enrique 214–3 × 544	11	17	35	63
Don Enrique INTA 320–5 × 711	0	10	49	59
Don Enrique INTA 322–1 × 711	40	8	19	67
Grand Crouelle 216–1 × 544	90	0	0	90
Grand Crouelle 217–3 × 544	92	0	0	92
Jaroslavna 251–1 × 544	86	0	0	86
Jaroslavna 251–3 × 544	46	0	0	46
Kalinka 249–1 × 544	46	0	0	46
Kalinka 249–4 × 544	21	0	0	21
Kartano 245–2 × 544	65	0	1	66
Kartano 246–3 × 544	60	0	0	60
Kisvardai 224–1 × 544	64	0	0	64
Kisvardai 224–3 × 544	89	0	0	89
Kungs 280–2 × 544	35	0	0	35
Kungs 280–3 × 544	40	0	0	40
Kustro 266–1 × 544	32	0	0	32
Kustro 266–3 × 544	23	0	0	23

Table 6

Male fertility of F_1 hybrids between male sterile BC_2 and/or BC_3 progenies and the L_1 inbred line (continued)

Source of male sterile BC_1 plants	Number of F_1 plants			
	MF	PF	MS	Total
L18 × 544	40	0	0	40
Madar 236–2 × 544	17	0	0	17
Madar 236–3 × 544	20	0	0	20
Maksimirska 226–3 × 544	80	0	0	80
Maksimirska 226–4 × 544	44	0	0	44
Merkator 283–1 × 544	38	0	0	38
Merkator 284–2 × 544	39	0	0	39
Ot1–3 × 544	43	0	0	43
Pasteoro Massaux 324–2 × 711	0	0	46	46
Pasteoro Massaux 324–4 × 711	0	0	54	52
Pico Gentario 328–3 × 711	28	0	11	39
Pico Gentario 330–2 × 711	20	0	10	30
Pico Mag 333–3 × 711	0	0	46	46
Pico Mag 334–1 × 711	23	0	9	32
Pico Urugway 230–2 × 544	4	5	85	94
Pico Urugway 230–4 × 544	1	8	52	61
Rheidol 233–1 × 544	67	0	0	67
Rheidol 233–5 × 544	88	0	0	88
Saratovskaja 259–3 × 544	34	0	0	34
Saratovskaja 259–6 × 544	26	0	0	26
Smolickie 274–1 × 544	40	0	0	40
Tschermaks Marchfelder 273–4 × 544	37	0	0	37
Tschermaks Marchfelder 273–6 × 544	38	0	0	38
Tulunskaja Zelenozernaja 256–2 × 544	40	0	0	40
Tulunskaja Zelenozernaja 256–3 × 544	24	0	0	24
Vjatka 2 262–1 × 544	41	0	0	41
Vjatka 2 262–5 × 544	34	0	0	34
Warko 234–2 × 544	43	0	0	43
Warko 235–1 × 544	80	0	0	80
Wibro 254–1 × 544	86	0	0	86
Wibro 254–2 × 544	57	0	0	57

heterozigosity in BC_2 and BC_3, it still causes some doubts. In order to get reliable evidence of the presence of Pampa cytoplasm in segregating progenies, male sterile F_1 segregants were crossed back to the L1 line. The resulting BC_1 and BC_2 progenies were male sterile as is shown in Table 7. Thus, 11 progenies (sources) appeared to have the *cms*–P cytoplasm. All of them originated from a single plants randomly taken from 6 South–American populations.

Table 7

Male fertility of backcross habrids from crosses between male sterile F_1 segregates and the L1 male parent

Cross	Number of plants							
	BC_1				BC_2			
	MF	PF	MS	Total	MF	PF	MS	Total
(Don Enrique 214–3 × 544)Bc3 × L1	0	0	57	57	0	4	59	63
(Pico Urugway 230–2 × 544)Bc3 × L1	0	1	55	56	0	0	34	34
(Pico Urugway 230–4 × 544)Bc3 × L1	0	0	61	61	0	2	32	34

DISCUSSION

No results of an effort to sample systematically the various populations of rye throughout the world for presence of sterility–inducing cytoplasms have thus far been published, but there is an ample evidence of widespred existence of such cytoplasms in cultivated, and possibly, in weedy rye species as well. References relevant to a number of separate discoveries of sterility–inducing cytoplasm are specified above, in the introductory section of the paper. One important fact about these discoveries should be mentioned here. All sources were identified initially by making sib – or out–pollination on spontaneous male sterile segregants found in different populations, with the exception of the 2 sources detected in intra–specific and inter–specific crosses. These two cases were described by Geiger and Schnell (1970) and Łapiński (1972), respectively. This paper summarise data pertinent to the occurrence and distribution of sterility–inducing and nonsterility–inducing cytoplasm as well, which were collected in Szczecin during last 15 years in effect of systematic testing of plants, lines and populations for the kind of cytoplasm present. Conventional but feasible methods of testing, based on plasmotype by genotype interaction were used throughout the work.

All of the representatives (single plants) of wild rye *S .anatolicum*, *S. kuprijanovii*, *S. kuprijanovii var. chaldicum* and *S. montanum*, currently thought to be a members of one species *S. montanum* (Kobyljanskij 1982), *S. silvestre* and *S. vavilovii* (*S .iranicum* Kobyl.) had nonsterility–inducing, i.e., normal cytoplasm. This is consistent

with an earlier findings of Łapiński (1972, 1973, 1977). In his investigations, pollen sterility differences between reciprocal crosses of wild species with cultivated rye led to the discovery of normal cytoplasm in *S. montanum* and sterilizing cytoplasm in *S. cereale*. No evidence to the contrary exists. Also *S. afghanicum, S. ancestrale* and *S. segetale*, a weedy–rye species included recently by Kobyljanskij (1982) into the *S. cereale* ssp. *vavilovii,* were found to be free of plants with sterility–inducing cytoplasm. Nevertheless, conclusions do not seem straightforward. Our inbred line DS2 appeared to have sterility–inducing cytoplasm and, to our knowledge, this cytoplasm was originally derived from *S. dighoricum*, another weedy – rye member of the *subspecies vavilovii.* Łapiński (1970) found male sterile segregant in an original sample of *S. dighoricum* plants. Furthermore, Kobyljanskij *et al.* (1994) observed the highest frequency of male sterile segregants in an old landrace cultivars and also in an unnamed populations of weedy – rye. Even though the last two pieces of information constitute only indirect evidence of the presence of sterility–inducing cytoplasm in weedy–ray populations it should be taken into account when conclusions are considered.

On the other hand, all of the evidence available indicates that sterility–inducing cytoplasm predominates in cultivated forms of *S. cereale*, but normal cytoplasm also occurs in those with considerable frequency. It is difficult or imposible to find any rule in the distribution of sterility–inducing cytoplasm. The cytoplasm inducing male sterility is wide–spread over continents, environments and countries, although it seems to be particularily frequent in European open–pollinated cultivars tracing back their origin to Petkus rye. If the last mention is true or false, it remains unproven, however, because of insufficient sampling.

Observation of the adequate test crosses revealed that 82% of newly "made to order" sources of cytoplasmic male sterility were identical in reaction to the Vavilovii cytoplasm; the remainder were of the Pampa type cytoplasm. It was imposible, of course, to detect any other type of sterility–inducing cytoplasm with the use of only one selected tester, even though it actually existed. Whether the new sources are exactly of Pampa and of Vavilovii type or Pampa–like and Vavilovii–like types, it remains unknown. Careful examination may some day reveal differences between sources of cytoplasm that presently seem to be identical, as it happened for example, in corn and some other crops (Kaul 1988). Unlike Kobyljanskij *et al.* (1994) and Warzecha and Warzecha (1996), we found the Pampa or Pampa – like cytoplasm only in South – American populations. Virtually all sources derived from European populations or from populations of European descend appeared to belong to the Vavilovii or Vavilovii – like type of sterility–inducing cytoplasm, as have been expected. Unfortunately, none of the populations studied is of Near East origin and testing of Turkish populations is not yet com–

pleted. Geiger and Miedaner (1996) reported on high frequency of strong restorer genes for Pampa cytoplasm in Argentinean, Turkish and Iranian populations. Obviously, this implies also the presence of Pampa or Pampa – like cytoplasm. Geiger's assumption have been confirmed by studies we have made with 6 south–American populations. Indeed, all these populations contain Pampa or Pampa – like cytoplasm at high frequency. One significant fact about occurrence of different cytoplasms within population should be mentioned here. All three cytoplasm, i.e., normal, Vavilovii and Pampa may be found in one population, and this was the case in current study. Within Argentinean 'Don Enrique' population both types of sterility inducing cytoplasm together with normal cytoplasm were detected.

CONCLUSIONS

Taking into account all relevant data available it can be safely stated that:

1. Male sterility–inducing cytoplasm is of frequent occurence in world–wide populations of cultivated rye while normal cytoplasm is relatively rare. Normal cytoplasm predominates in wild and , possibly, in weedy rye populations while a cytoplasm inducing male sterility is lacking and rare, respectively.
2. With regard to their internal plasmotypic composition an open–pollinated populations of cultivated rye may be classified into three groups: a) homogeneous, composed of plants carrying normal cytoplasm, plants carrying sterility–inducing cytoplasm are lacking or rare; B) homogeneous, composed of plants carrying sterilizing cytoplasm, plants carrying normal cytoplasm are lacking or rare; c) heterogeneous, composed of both plant types, carrying sterilizing and normal cytoplasm.
3. The male sterility–inducing Vavilovii cytoplasm prevails in open–pollinated populations of cultivated rye. The Pampa type of sterility–inducing cytoplasm is lacking or unique in populations of European descend but it occurs quite frequently in South–American populations.

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