Theor Appl Genet (2006) 112: 627–633
DOI 10.1007/s00122-005-0164-3

ORIGINAL PAPER

Stefan Stojałowski · Mirosław Łapiński
Marek Szklarczyk

Identification of sterility-inducing cytoplasms in rye using the plasmotype–genotype interaction test and newly developed SCAR markers

Received: 28 January 2005 / Accepted: 13 November 2005 / Published online: 7 December 2005

Abstract A series of 25 rye (*Secale cereale* L.) inbred lines was tested with respect to three mitochondrial sequence-characterized amplified region (SCAR) polymorphisms. The analysis revealed a close association between the marker-determined mitotypes and plasmotypes (cytoplasm types known from breeding data) represented by the inbreds. The mitochondrial markers also confirmed normal (N-) cytoplasmic character of three wild rye species: *Secale montanum*, *S. vavilovii* and *S. kuprijanovii*. For 186 plants from open-pollinated cultivars of Turkish and South American origin, cytoplasm identification was performed with the use of crossing with double non-restoring tester. In 77 plants the normal (N) cytoplasm was detected, and for 63 of these the PCR analysis was performed producing results which were consistent with the genetic data based on testcrossing and phenotype assessment. The mitochondrial markers also confirmed a presence of sterility-inducing cytoplasm in the remaining 109 plants. Moreover, the markers allowed for differentiation between Pampa (P-) and Vavilovii (V-) cytoplasmic individuals. For 60 plants the latter results were verified using crosses with a line maintaining P-cytoplasmic sterility and acting as a restorer of the V-cytoplasm. For two of these plants contradicting results were produced with the applied methods of cytoplasm identification and the basis of this discrepancy is discussed. Regardless of the identification method, widespread occurrence of a sterility-inducing cytoplasm was revealed, especially in South American populations.

Communicated by H. H. Geiger

S. Stojałowski (✉) · M. Łapiński
Department of Genetics and Plant Breeding,
Agricultural University of Szczecin, Słowackiego 17,
71-434 Szczecin, Poland
E-mail: stefs@agro.ar.szczecin.pl
Tel.: +48-91-4250259
Fax: +48-91-4871962

M. Szklarczyk
Department of Genetics, Plant Breeding and Seed Science,
Agricultural University of Kraków, 29 Listopada 54,
31-425 Kraków, Poland

Introduction

In rye (*Secale cereale* L.) two genetically different types of cytoplasmic male-sterility (CMS) are known: Pampa and Vavilovii. Pampa (P-) cytoplasm was discovered by Geiger and Schnell (1970) in Argentinean population of rye, and at present it is commonly exploited for hybrid breeding purposes. Vavilovii (V-) cytoplasm is represented by numerous sources of which the best known are: R, found in Russian populations (Kobyljanskij 1971), C (Łapiński 1972) and S (Madej 1975), both detected in Polish rye Smolickie as well as G, originating from cultivar Schlägler Alt (Adolf and Winkel 1985). Due to lack of effective maintaining genotypes the use of V-cytoplasms in hybrid seed production is rather limited. The respective non-restorer genes were probably eliminated from cultivated populations, which quite frequently contain the V-cytoplasm (Łapiński and Stojałowski 2003).

Classical cytoplasm identification is based on the test of interaction between plasmotype and genotype. Examined plants are pollinated with a proper tester line and subsequently the resulted progenies are evaluated in respect of their sterility/fertility phenotype. In case of rye, this procedure takes at least 2–3 growth seasons. However, with the advent of molecular methods, time- and labor-consuming classical testing can be substituted with the use of DNA markers. Male sterility caused by P-cytoplasm is associated with changes in the mitochondrial genome (Tudzynski et al. 1986; Dohmen and Tudzynski 1994; Dohmen et al. 1994). MtDNA profiling also revealed differences between G, P and normal (N) cytoplasm (Steinborn et al. 1993). All these reports are based on an RFLP approach which is hardly suitable in high-throughput genotyping. For convenience of routine analysis PCR-based markers are developed and due to

their simplicity large populations can be screened quickly and at a low cost. Cytoplasmic PCR markers were identified for several plant species, e.g. onion (Havey 1995; Engelke et al. 2003) and carrot (Szklarczyk et al. 2000). So far, such markers have not been reported for rye. On the basis of rye mitochondrial sequence data we designed three markers of this type. Here, we demonstrate their usefulness to discriminate between different cytoplasm sources of rye.

Materials and methods

Plant material

Most of the tested inbreds, including alloplasmic versions of line 544 (Łapiński and Stojałowski 1996), were developed in the Department of Genetics and Plant Breeding of the Agricultural University of Szczecin, Poland. Lines C599 and S436N were produced in DANKO Plant Breeding Ltd., Choryń, Poland. Lines NS857P/95, NS714P/1/00, NS839P/00, NS310P/89 and WS155P/96 were obtained from Poznań Plant Breeding Ltd., Tulce, Poland. Line L1 originated from the University of Hohenheim, Germany. The seed of wild rye species *Secale montanum* Guss., *S. vavilovii* Grossh. and *S. kuprijanovii* Grossh. was obtained from Botanical Garden, Center for Biological Diversity Conservation of the Polish Academy of Sciences, Warsaw-Powsin, Poland. Seed samples of open-pollinated cultivars were received from the National Centre of Plant Genetic Resources located in Plant Breeding and Acclimatization Institute, Radzików, Poland.

Plasmotype–genotype interaction (PGI) test

The PGI test was carried out for the purpose of cytoplasm identification. Pollination with double non-restorer line L711 was used to distinguish between normal and sterilizing cytoplasm. Fertility/sterility phenotype was visually evaluated according to Geiger and Morgenstern (1975) in either BC_1–BC_2 (Turkish populations) or BC_1–BC_3 (South American populations) progenies. The type of sterilizing cytoplasm was determined using line L1 as a tester. This inbred is known as non-restoring for CMS-Pampa and restoring for cytoplasms of the V group. Details of the PGI test are given by Łapiński and Stojałowski (2003).

DNA isolation

For the inbred lines and wild species total genomic DNA was extracted from leaves harvested at tillering stage. The isolation procedure was performed using Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland). For cytoplasm identification in cultivars we used DNA from the respective BC_2 or BC_3 progenies originating from the cross with line L711 (see previous). In this case template

Table 1 Mitochondrial SCAR markers identified for a series of rye inbreds and three wild rye species

Inbred line	Cytoplasm	Marker		
		cox1 0.89/0.93 kb	*nad6* 0.85 kb	*nad2* 0.80 kb
544(N)	N	−/+	−	−
544(CMS-P)	P	+/−	+	−
544(CMS-R)	V	−/+	(+)	+
544(CMS-S)	V	−/+	−	+
544(CMS-C)	V	−/+	−	+
Ot0–20	V	−/+	−	+
Ot1–3	V	−/+	−	+
DS2	V	−/+	−	+
RXL10	N	−/+	−	−
RoP2	N	−/+	−	−
L1	N	−/+	−	−
S436N	P	+/−	+	−
620/75-1-5	N	−/+	−	−
C599	N	−/+	−	−
KaH6	N	−/+	−	−
542-9N	N	−/+	−	−
153/79	N	−/+	−	−
585/92-1-2	N	−/+	−	−
585/92-6-1	N	−/+	−	−
585/92-6-6	N	−/+	−	−
NS857P/95	P	+/−	+	−
NS714P/1/00	P	+/−	+	−
NS839P/00	P	+/−	+	−
NS310P/89	P	+/−	+	−
WS155P/96	P	+/−	+	−
S. montanum Guss.	N	−/+	−	−
S. vavilovii Grossh.	N	−/+	−	−
S. kuprijanovii Grossh.	N	−/+	−	−

+ Fragment present
− Fragment absent
( ) Weak amplification

DNA was isolated from young leaves according to the single-step method described by Thomson and Henry (1995).

DNA amplification

The 15 µl of the PCR mixture contained: 1.5 µM dNTPs, 1.5 mM $MgCl_2$, 5 pmol of each primer, 0.8 U of Taq DNA polymerase (recombinant, MBI Fermentas), either 1- or 0.15-fold ammonium sulphate PCR buffer (MBI Fermentas) and template DNA. The latter buffer concentration was used only if template DNA originated from Thomson and Henry (1995) procedure and was already dissolved in buffered solution (0.1 M Tris–HCl, 1 M KCl, 10 mM EDTA). The amount of DNA in the mixture also depended on the extraction method: 10 ng for template isolated with Genomic Mini Kit (see previous) and 3 ng for DNA from the procedure of Thomson and Henry (1995).

The following primer pairs were used for PCR (5′–3′): cox1-F: TACTTCACCGCAGCTACCAT/cox1-R: AGTCCAGTCTTGTCACTTTC, nad6-F: TTCAAATA GCGGAGATTCAC/nad6-R: ATGCTATAA CCA GACCATAG and nad2-F: TTCCATGATCTATG GGTCTA/nad2-R: GATATCGTAAGCCATAATAG.

They were used to generate markers based on *cox1*, *nad6* and *nad2* gene, respectively. In each pair the forward (F) primer is anchored within coding sequence of the indicated gene, while the reverse (R) one anneals in the adjacent 3′ flanking region. The respective sequence information was submitted to the EMBL database under accessions no. AM050160, AM050161, AM050162 and AM050163. Exact primer positions are indicated in the sequence records.

The reactions were carried in Eppendorf Mastercycler Gradient, programmed as follows: 5 min of initial denaturation in 94°C; 35 cycles of: 92°C for 45 s, 57°C for 45 s and 72°C for 90 s followed by final elongation in 72°C for 10 min. PCR products were resolved in 1.5% agarose gel containing ethidium bromide at a concentration of 0.4 µg/ml. Gels were run in TBE buffer at 4 V/cm for 2 h and examined in UV light.

Results

At first, the PCR analysis was performed for a set of 25 inbreds with plasmotypes known either on the basis of origin or formerly determined by Łapiński and Stojałowski (2003) with the use of plasmotype/genotype interaction test (Table 1). Generally, the results of PCR were in agreement with the genetic data. It appeared that the given cytoplasm type was associated with a specific combination of the respective mitochondrial products.

For lines 544P, S436N, NS857P/95, NS714P/1/00, NS839P/00, NS310P/89 and WS155P/96 possessing P-cytoplasm, marker *cox1* produced a fragment of 0.89 kb (Table 1). In case of lines representing normal cytoplasm or cytoplasms of the V group this marker revealed a distinctly longer (0.93 kb) DNA fragment.

Use of *nad6* marker yielded a product of 0.85 kb. It was observed for all lines with the P-cytoplasm as well as for line 544R representing Russian CMS source of the V type (Fig. 1). However, in the latter case amplification was very weak. For the remaining inbreds this primer pair did not produce any DNA fragments.

In case of *nad2* marker amplification was observed only for lines with cytoplasm from the V group. The respective product was 0.8 kb long.

The above set of mitochondrial markers was subsequently applied to wild species of rye: *Secale montanum*, *S. vavilovii* and *S. kuprijanovii* (Table 1). For all three forms amplification was observed only in case of *cox1* marker—it yielded a fragment of 0.93 kb. Therefore, mitotype of the examined *Secale* species was identical to that of N-cytoplasmic inbreds (see previous).

In the next step both the PGI and PCR testing were applied to examine plants originating from eight open-pollinated cultivars. Crossing with double non-restorer line L711 allowed determining whether these plants had normal or sterilizing cytoplasm (Table 2). For 77 plants substitution backcrossing resulted in fully male-fertile progenies indicating the presence of normal (N) cytoplasm. The PCR analysis was performed for 63 of these plants representing Turkish populations. According to expectations amplification was observed only in case of *cox1* marker revealing the presence of 0.93 kb amplicon.

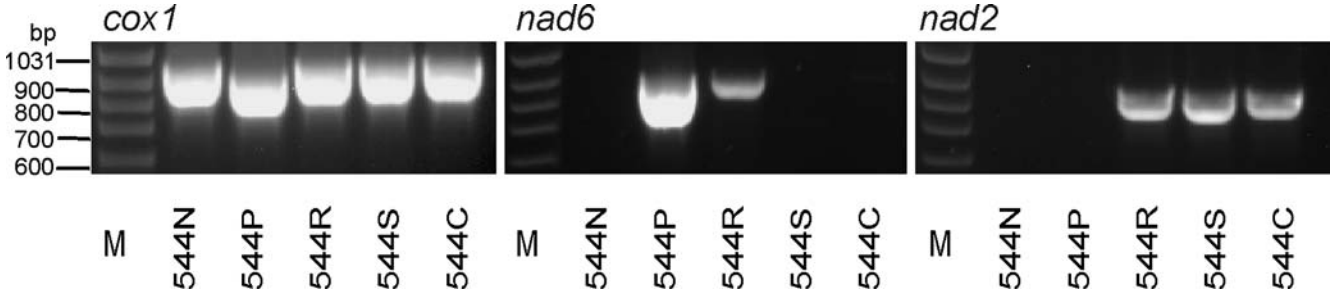

Fig. 1 PCR products of the mitochondrial SCAR markers derived from genes *cox1*, *nad6* and *nad2* obtained for a set of alloplasmic versions of line 544, *M* DNA size standard (100 bp DNA Ladder Plus, MBI Fermentas)

Table 2 Distribution of normal and sterilizing cytoplasm in rye populations as determined by crossing with line L711 (PGI test) and the use of mitochondrial SCAR markers

Population	No. of tested plants	No. of plants with normal cytoplasm indicated by		No. of plants with sterilizing cytoplasm indicated by	
		PGI test	SCAR markers	PGI test	SCAR markers (CMS-P/CMS-V)
Populations from Turkey					
Candar	24	13	13	11	0/11
Ancora	24	24	24	0	0/0
Turkey 75	20	19	19	1	0/1
Harlan I.R.6982	26	7	7	19	0/19
Populations from South America					
Don Enrique INTA	24	0	–	24	24/0
Pasteoro Massaux	23	10	–	13	5/8
Pico Gentario	22	1	–	21	21/0
Pico Mag	23	3	–	20	17/3
Total	186	77	63	109	67/42

Line L711 is non-restoring for both Pampa and Vavilovii cytoplasm
– Not tested with the use of SCAR markers

An increasing proportion of plants with normal cytoplasm was found within: Harlan I.R.6982, Candar and Turkey 75, respectively. The studied sample of Ancora appeared to be entirely N-cytoplasmic. The remaining 14 plants with N-cytoplasm originated from South American material, mostly from Pasteoro Massaux, and these were not tested with the SCAR markers. Backcross progenies of the remaining 109 individuals were either fully male-sterile (MS) or segregated to male-fertile and male-sterile plants with the significant proportion of the latter. Due to the non-restoring genotype of the tester line, this result indicated the presence of sterilizing cytoplasm. Application of the SCAR markers (Fig. 2) allowed for more precise cytoplasm identification in these plants—according to mitotypes 67 were found to be P-cytoplasmic, in the remaining 42 V-cytoplasm was detected. Pampa was found only in South American populations, in which it was represented by a majority of the analyzed plants. The sterilizing cytoplasm present in the studied Turkish material was identified as of V-type.

Furthermore, from the pool of 109 progenies (see previous) we randomly chose 60 from which male-sterile plants were subjected to further examination. They represented either BC_2 or BC_3 hybrid generations derived from 4 South American to 2 Turkish cultivars. In order to determine what type of sterility-inducing cytoplasm these plants possessed we crossed them with line L1 acting as a non-restorer for Pampa and as a restorer for V-cytoplasm. Therefore, occurrence of male-sterile plants in the resulted F_1 offspring indicated the presence of P-cytoplasm, whereas lack of those implied the presence of V plasmotype. The results of these crosses were compared with data generated using PCR analysis (Table 3). In 58 cases discrimination between P- and V-cytoplasm yielded consistent results for both methods of testing. Among those, the majority of South American material carried P-cytoplasm, while the Turkish CMS sources were assigned to V type. However, for another two plants, one from Pasteoro Massaux and the other from Candar, conflicting data were obtained with these methods. The use of conventional testing assigned both plants to P-type, whereas the SCAR markers indicated the presence of V-like mitotype.

Discussion

In rye crossing with selected pollen donors provides relatively an easy way either to differentiate between normal and sterility-inducing cytoplasm or to determine the type of sterilizing cytoplasm. However, this method of identification is both labor- and time-consuming, mostly because at least 2–3 hybrid generations are required. Over 10 years ago Steinborn et al. (1993) reported polymorphic mitochondrial RFLPs, which allowed for differentiation between N-, P- and V-plasmotypes. Nevertheless, for routine use in the breeding practice PCR-based markers offer a much better alternative to the RFLP approach. In addition to overall simplicity, PCR requires less DNA, and poor template quality is often tolerated. Therefore, in many cases even very simplified preparation procedures work well enabling for a fast and a reliable analysis on the population scale. Our assay system for cytoplasm identification in rye consists of three SCAR markers although in principle two of them, *cox1* and *nad2*, provide sufficient discrimination. The marker based on *nad6* sequence has rather confirmatory character. In general, its product is amplified only for plants carrying the P-cytoplasm. However, weak amplification was also observed for CMS-R source of the V-type making us less confident about the diagnostic value of this marker. Therefore, we recommend to always include the marker based on *cox1* sequence. The latter also provides a positive control for N-cytoplasmic plants which do not yield any product in case of either *nad2* or *nad6* assay.

The main focus of our research was to compare results of conventional cytoplasm identification with data produced with the use of our mitochondrial marker set. Out of 200 available paired comparisons (Tables 1, 2, 3),

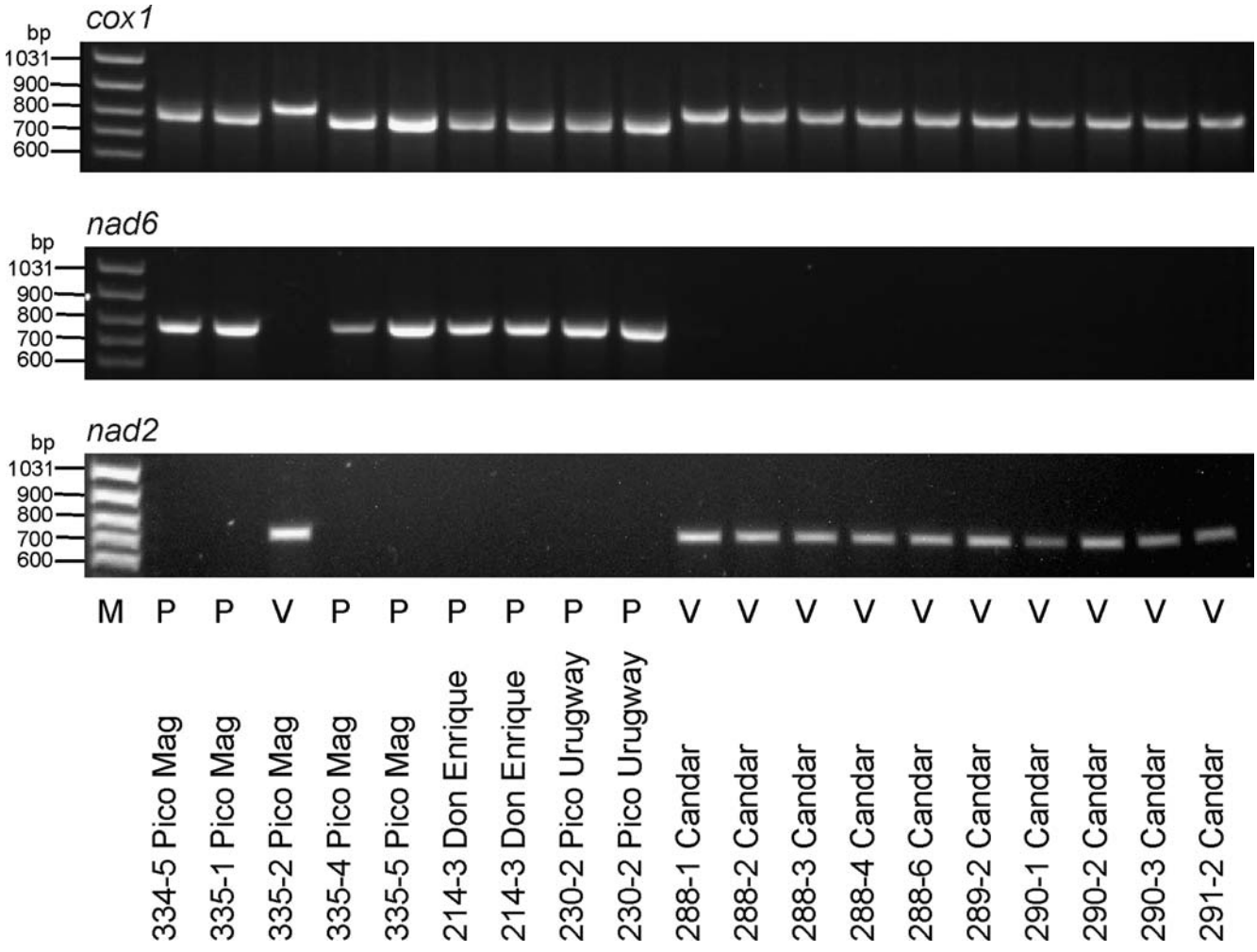

Fig. 2 PCR products of the mitochondrial SCAR markers derived from genes *cox1*, *nad6* and *nad2* obtained for rye plants originating from different populations, deduced cytoplasm type specified below the respective lane, *M* DNA size standard (100 bp DNA Ladder Plus, MBI Fermentas)

only two cases of inconsistency between both methods were revealed. Contradicting indications were produced for single plants from Pasteoro Massaux and Candar. According to the PGI test they both carried P-cytoplasm, but SCAR markers indicated that instead the V-cytoplasm was present. We cannot exclude that this inconsistency is a consequence of some technical error which might have occurred in the course of conventional testing, based in this case on only one hybrid generation. However, such disagreement is also likely to result from physical distinctness of molecular CMS determinants and the sequences our markers are based on. According to this explanation, genetic data (conventional test) reflect the presence of the causal CMS factor, and its correlation with the observed mitotype has rather quantitative than absolute character, similar to the relation of linkage observed in nuclear genomes. This assumption implies that plants Pasteoro Massaux 327-1 and Candar 288-1 carry the form of Pampa which exhibits mitotype usually associated with the V-cytoplasm.

Our previous research showed that wild-rye species *Secale montanum*, *S. vavilovii* and *S. kuprijanovii* carried non-sterilizing cytoplasm, and also that the sterility-inducing cytoplasm was widely spread in populations of cultivated rye (Łapiński and Stojałowski 2003). These results acquired strong support with the use of our marker system. The presence of P-cytoplasm in South American rye agrees with the fact that Geiger and Miedaner (1996) detected strong restorer genes for the P-cytoplasm in the populations from this region. Reported here, broad occurrence of Pampa in South American material must have led to the selection pressure eliminating the non-restorer alleles. Up to now, with the exception of DZ-1 source (Warzecha and Salak-Warzecha 1996), the P-cytoplasm has not been found in populations other than South American. However, effective restorer genes for Pampa were also discovered in populations of Near East origin (Geiger and Miedaner 1996) indicating that P-cytoplasm may also be present in rye from this region. In our study the Near East material was represented by populations from

Table 3 Identification of sterilizing cytoplasm (CMS-P vs. CMS-V) in rye plants from different populations by means of crossing with line L1 (PGI test) and the mitochondrial SCAR markers

Plant (with the source population indicated)	No. of plants in F_1 progeny			Marker			Cytoplasm (PGI/SCAR)
	MF	PF	MS	*cox1* 0.89/0.93 kb	*nad6* 0.85 kb	*nad2* 0.80 kb	
Don Enrique 214-3	11	17	35	+/−	+	−	P/P
Don Enrique INTA 320-5	0	10	49	+/−	+	−	P/P
Don Enrique INTA 320-6	0	0	59	+/−	+	−	P/P
Don Enrique INTA 321-1	0	0	55	+/−	+	−	P/P
Don Enrique INTA 321-2	0	5	7	+/−	+	−	P/P
Don Enrique INTA 321-3	3	0	55	+/−	+	−	P/P
Don Enrique INTA 322-1	40	8	19	+/−	+	−	P/P
Don Enrique INTA 322-2	3	0	49	+/−	+	−	P/P
Don Enrique INTA 323-3	16	5	8	+/−	+	−	P/P
Don Enrique INTA 322-4	0	0	53	+/−	+	−	P/P
Don Enrique INTA 322-5	10	0	51	+/−	+	−	P/P
Don Enrique INTA 323-2	10	0	47	+/−	+	−	P/P
Don Enrique INTA 323-5	12	10	59	+/−	+	−	P/P
Pasteoro Massaux 324-1	56	0	0	−/+	−	+	V/V
Pasteoro Massaux 324-2	0	0	46	+/−	+	−	P/P
Pasteoro Massaux 324-4	0	0	54	+/−	+	−	P/P
Pasteoro Massaux 325-5	0	0	49	+/−	+	−	P/P
Pasteoro Massaux 326-1	60	0	0	−/+	−	+	V/V
Pasteoro Massaux 326-4	0	0	58	+/−	+	−	P/P
Pasteoro Massaux 327-1	**2**	**0**	**17**	**−/+**	**−**	**+**	**P/V**
Pasteoro Massaux 327-3	53	0	0	−/+	−	+	V/V
Pasteoro Massaux 327-5	0	0	54	+/−	+	−	P/P
Pico Gentario 328-2	6	0	10	+/−	+	−	P/P
Pico Gentario 328-3	21	8	10	+/−	+	−	P/P
Pico Gentario 329-4	2	10	10	+/−	+	−	P/P
Pico Gentario 329-5	0	0	60	+/−	+	−	P/P
Pico Gentario 329-6	0	0	62	+/−	+	−	P/P
Pico Gentario 330-1	0	0	52	+/−	+	−	P/P
Pico Gentario 330-2	19	0	11	+/−	+	−	P/P
Pico Gentario 330-3	0	0	61	+/−	+	−	P/P
Pico Gentario 330-4	0	0	60	+/−	+	−	P/P
Pico Mag 332-5	61	0	0	−/+	−	+	V/V
Pico Mag 332-6	8	5	5	+/−	+	−	P/P
Pico Mag 333-1	0	0	68	+/−	+	−	P/P
Pico Mag 333-3	0	0	46	+/−	+	−	P/P
Pico Mag 334-1	23	0	9	+/−	+	−	P/P
Pico Mag 334-3	10	0	12	+/−	+	−	P/P
Pico Mag 334-5	13	0	12	+/−	+	−	P/P
Pico Mag 335-1	0	0	40	+/−	+	−	P/P
Pico Mag 335-2	29	0	0	−/+	−	+	V/V
Pico Mag 335-3	1	1	55	+/−	+	−	P/P
Pico Mag 335-4	0	1	57	+/−	+	−	P/P
Pico Mag 335-5	0	0	51	+/−	+	−	P/P
Pico Mag 335-6	0	0	56	+/−	+	−	P/P
Harlan I.R.6982 300-1	28	0	0	−/+	−	+	V/V
Harlan I.R.6982 300-3	42	0	0	−/+	-	+	V/V
Harlan I.R.6982 300-4	67	0	0	−/+	−	+	V/V
Harlan I.R.6982 300-5	48	0	4	−/+	−	+	V/V
Harlan I.R.6982 301-2	24	0	3	−/+	−	+	V/V
Harlan I.R.6982 302-1	55	0	0	−/+	−	+	V/V
Harlan I.R.6982 302-3	50	0	0	−/+	−	+	V/V
Harlan I.R.6982 302-5	49	0	0	−/+	−	+	V/V
Harlan I.R.6982 302-8	63	0	2	−/+	−	+	V/V
Harlan I.R.6982 303-1	53	1	1	−/+	−	+	V/V
Harlan I.R.6982 303-5	59	0	0	−/+	−	+	V/V
Candar 288-1	**0**	**0**	**16**	**−/+**	**−**	**+**	**P/V**
Candar 288-4	46	0	0	−/+	−	+	V/V
Candar 289-2	49	0	0	−/+	−	+	V/V
Candar 290-1	41	0	0	−/+	−	+	V/V
Candar 290-2	45	0	0	−/+	−	+	V/V

Line L1-non-restoring for Pampa but restorer of Vavilovii cytoplasm*MF* male-fertile, *MS* male-sterile, *PF* partially fertile; *bold* marks the plants for which contradicting results were obtained
+ Fragment present
− Fragment absent

Turkey, in which mostly N- and V-cytoplasmic plants were found. The only exception mentioned is Candar 288-1 for which contradicting indications were produced (see previous).

General consistency of the two methods we used for cytoplasm identification shows that newly developed SCAR markers provide efficient alternative to laborious and time-consuming genetic testing. These markers can be easily adapted to rye breeding practice facilitating selection of parental components. They will be particularly useful in early stages of selection process when appropriate genotypes are being extracted from open-pollinated populations of mixed cytoplasmic composition. PCR analysis of the mitochondrial polymorphisms can also assist in restorer breeding programs, when contamination by N-cytoplasm can lead to selection of fertile plants lacking *Rf* alleles.

Acknowledgments This study was supported by Polish State Committee for Scientific Research (grant no. 3 P06A 041 22). The experiments comply with the current laws of Poland.

References

Adolf K, Winkel A (1985) A new source of spontaneous sterility in winter rye—preliminary results. In: Proceedings of Eucarpia meet cereal sect on rye, Svalöv, Sweden, pp 293–306

Dohmen G, Tudzynski P (1994) A DNA-polymerase-related reading frame (*pol-r*) in the mtDNA of *Secale cereale*. Curr Genet 25:59–65

Dohmen G, Hesseberg H, Geiger HH, Tudzynski P (1994) CMS in rye: comparative RFLP and transcript analyses of mitochondria from fertile and male-sterile plants. Theor Appl Genet 89:1014–1018

EngelkeT, Terefe D, Tatlioglu T (2003) A PCR-based marker system monitoring CMS-(S), CMS-(T) and (N)-cytoplasm in the onion (*Allium cepa* L.). Theor Appl Genet 107:162–167

Geiger HH, Miedaner T (1996) Genetic basis and phenotypic stability of male-fertility restoration in rye. Vortr Pflanzenzüchtg 35:27–38

Geiger HH, Morgenstern K (1975) Angewandt-genetische Studien zur cytoplasmatischen Pollensterilität bei Winterroggen. Theor Appl Genet 46:269–276

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

Havey MJ (1995) Identification of cytoplasms using the polymerase chain reaction to aid in the extraction of maintainer lines from open-pollinated populations of onion. Theor Appl Genet 90:263–268

Kobyljanskij VD (1971) Production of sterile analogues of winter rye varieties, sterile maintainers and fertility restorers. Trudy Prikl Bot Genet Sel 44:76–85

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

Łapiński M, Stojałowski S (1996) The C-source of sterility-inducing cytoplasm in rye: origin, identity and occurrence. Vortr Pflanzenzuecht 35:51–60

Łapiński M, Stojałowski S (2003) Occurrence and genetic identity of male sterility-inducing cytoplasm in rye (*Secale* spp.). Plant Breed Seed Sci 48:7–23

Madej L (1975) Research on male sterility in rye. Hod Rośl Aklim i Nasien 19:421–422

Steinborn R, Schwabe W, Weihe A, Adolf K, Melz G, Boerner T (1993) A new type of cytoplasmic male sterility in rye (*Secale cereale* L.): analysis of mitochondrial DNA. Theor Appl Genet 85:822–824

Szklarczyk M, Oczkowski M, Augustyniak H, Börner T, Linke B, Michalik B (2000) Organisation and expression of mitochondrial *atp9* genes from CMS and fertile carrots. Theor Appl Genet 100:263–270

Thomson D, Henry R (1995) Single-step protocol for preparation of plant tissue for analysis by PCR. BioTech 19:394–400

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

Warzecha R, Salak-Warzecha K (1996) Comparative studies on CMS sources in rye. Vortr Pflanzenzüchtg 35:39–49