Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
http://dx.doi.org/10.5424/sjar/2014124-6144

Spanish Journal of Agricultural Research 2014 12(4): 1124-1130
ISSN: 1695-971X
eISSN: 2171-9292

RESEARCH ARTICLE

OPEN ACCESS

Male sterility of triticale lines generated through recombination of triticale and rye maintainers

Tomasz Warzecha*, Agnieszka Sutkowska and Halina Góral

University of Agriculture in Kraków. Department of Plant Breeding and Seed Science. Łobzowska str. No. 24, 31-140 Kraków, Poland

Abstract

The *Triticum timopheevi* cytoplasmic male sterility (cms) system in triticale (×*Triticosecale* Wittmack) suffers from a low frequency of maintainers and environmental instability of the male sterility. On the other hand, the Pampa cms system in rye (*Secale cereale*) exhibits strong male sterility and a low frequency of restorers. Here, we report generating hybrids between maintainers of the *T. timopheevi* cms system in triticale and maintainers of the rye Pampa cms system. Ten hybrids were obtained. Their hybridity was verified by PCR (polymerase chain reaction) using ISSR (inter simple sequence repeats) primers. The cms maintaining ability of F_2 individuals and their progeny was tested. The F_2 plants were crossed to male sterile lines of triticale carrying the *T. timopheevi* cytoplasm. Among 180 G_1 offspring of these crosses, 71 (39.4%) were completely male sterile. Fourteen F_2 individuals (7.8%), as well as their F_2S_1 and progeny, generated stable male sterility in G_1, G_1BC_1 and G_1BC_2 generations after the crosses. Our results suggest that it is possible to produce a more stable cms system in triticale based on the *T. timopheevi* cytoplasm as compared to the existing one.

Additional key words: cms systems; cytoplasm; *Triticum timopheevi*; Pampa; ISSR.

Introduction

Cultivars of triticale (×*Triticosecale* Wittmack) are pure line cultivars, in terms of their genetic structure, obtained by the pedigree breeding method. However, there has been a growing interest in breeding hybrid triticale. Breeding of hybrid cultivars requires controlled crossing of hybrid components in the last stage of the process. In triticale, similar to other small grain cereals, it is possible to use chemical emasculation of female lines or transform them into male sterile lines. The male sterility system to be used in triticale must include fully male sterile lines, as female and pollinators effective in fertility restoration in F_1 hybrids. Among several alien cytoplasm sources that cause pollen sterilizing effect in triticale, only *Triticum timopheevi* (Zhuk.) and *Aegilops sharonensis* cytoplasms appear useful for male sterility systems, since they do not compromise important agronomic traits (Nalepa, 1990). The *T. timopheevi* cytoplasmic male sterility (cms) system in triticale is characterized by a low frequency of maintainers (Warzecha *et al*., 1998; Góral & Spiss, 2005; Góral *et al*., 2007). Additionally, the male sterility phenotype is not stable across environments (Góral *et al*., 2006). Most triticale lines and cultivars substantially, albeit not fully, restore fertility

* Corresponding author: rrwarzec@cyf-kr.edu.pl
Received: 24-04-14. Accepted: 04-11-14

This work has one supplementary figure that does not appear in the printed article but that accompanies the paper online

Abbreviations used: AFLP (amplified fragment length polymorphism); BC_{13}, BC_{17} (backcross, digit indicates number of backcross); cms (cytoplasmic male sterility); DH (doubled haploids); F_1 (hybrid generation); F_2 (first segregating generation); F_2S_1, F_2S_2 (subsequent self-pollinated generations of F_2 generation); G_1 (generation originated from crosses of cms triticale line to F_2 individuals); G_1BC_1, G_1BC_2 (subsequent generations of backcrossing cms G_1 to male fertile progenies of F_2 generation); ISSR (inter simple sequence repeats); PCR (polymerase chain reaction); RAPD (random amplified polymorphic DNA); S_9, S_{13}, S_{17}, S_{24} (self-pollinated generation, digit indicates number of generation); SSR (single sequence repeat).

of the *T. timopheevi* cytoplasm (Góral & Spiss, 2005; Góral *et al*., 2007). Because of the low frequency of maintainers, a large number of cultivars and lines must be screened and germplasm development must be carefully planned.

Since wheat lines with *T. timopheevi* cytoplasm have a low frequency of restorer lines but triticale shows a high frequency of genotypes containing restorer genes, it has been suggested that genes restoring male fertility in triticale originate from the rye genome. This hypothesis comes from the Curtis & Lukaszewski (1993) study, which found that genes restoring male fertility in F_1 hybrids of hexaploid wheat possessing *T. timopheevi* cytoplasm are located on the long arm of rye chromosomes 6R and 4R. Nevertheless, the Pampa cms system (Geiger & Schnell, 1970) can produce complete and stable male sterility in rye. It was hypothesized that non-restoring genes functioning in rye in the Pampa cms system may act in a similar way when transmitted to triticale with the *T. timopheevi* cytoplasm.

The ISSR-PCR markers offer several advantages over other marker types, such as high polymorphism levels, relatively low demands on the quality and quantity of DNA, and no requirements for special PCR or electrophoresis conditions. Additionally, higher temperatures, compared for example to the random amplified polymorphic DNA (RAPD) technique that utilizes lower annealing temperatures, work better to reduce nonspecific amplification. Many authors who compared RAPD and ISSR markers concluded that ISSR markers showed higher polymorphism and reproducibility (Nagaoka & Ogihara, 1997; Carvalho *et al*., 2005; Liu *et al*., 2007). As suggested by Naresh *et al*. (2009) sometimes SSR markers are preferred over the dominant markers like RAPD, ISSR, and amplified fragment length polymorphism (AFLP) due to its co-dominant nature. Therefore, the dominant nature of ISSR markers is definitely a disadvantage of this type of markers.

But the ISSR markers were also used in the studies of Bianco *et al*. (2011) concerning purity of F1 populations generated by crossing different forms of artichoke including male sterile artichoke (MS6) and interspecific hybrids from the *Triticeae* tribe (Carvalho *et al*., 2005). Moreover ISSR markers reveal polymorphism of non-coding regions associated with microsatellite sequences but located between them. It means we get at the same time information about the presence or absence of microsatellite sequences as well as polymorphism between microsatellite sequences.

The objectives of this study were (i) to obtain hybrids between triticale maintainers of the *T. timopheevi* cms system (used as female) and rye maintainers of the Pampa cms system (used as male); (ii) to verify that the F_1 generation is indeed of hybrid origin using ISSR (inter simple sequence repeats) markers; and (iii) to examine if it is possible to obtain stable non-restorer lines for the *T. timopheevi* cms system through crossing triticale and rye.

Material and methods

Six crosses were made between two hexaploid triticale maintainers of the *T. timopheevi* cms system, which were used as female (Salvo, which was in the S_{17} generation and 19 in the S_{13} generation) and three rye (*Secale cereale* L.) maintainers of the Pampa cms system (541-6 in S_{24}, 585/92-6-1 in S_9, and 585/92-1-2 in S_9), which were used as male. Several fertile F_1 hybrids were obtained and their hybrid nature was verified with ISSR-PCR. Leaves of four F_1 hybrids and parental lines (Table 1) were used to isolate the genomic DNA utilizing the Plant Mini AX kit (A and A Biotechnology). The analyses were conducted with four ISSR primers: ISSR01 $(TC)_8C$, ISSR03 $(GGGTG)_3$, ISSR06 $(AC)_8G$ and ISSR07 $(AC)_8T$. Their sequence and amplification conditions came from the publication by Stepansky *et al*. (1999). The ISSR markers that we utilized are considered to give higher polymorphism and reproducibility since the higher temperatures that we used, compared to the RAPD PCR method, reduce nonspecific amplification when compared for example to RAPD markers (Nagaoka & Ogihara, 1997; Carvalho *et al*., 2005; Liu *et al*., 2007). PCR was carried out using a Gene Amp 2400 thermal cycler

Table 1. Number of F_1 hybrids generated from crosses between male sterility maintainers of triticale and rye

Triticale line	Rye line	No. of ears	No. of flowers	No. of F_1 grains
Salvo 15/1*	541-6	21	876	3*
	585/92-6-1	65	2912	2
	585/92-1-2	21	924	0
19*	541-6*	15	840	1*
	585/92-6-1*	12	722	1*
	585/92-1-2*	17	908	3*
Sum		151	7182	10

* Individuals for which molecular analyses were performed

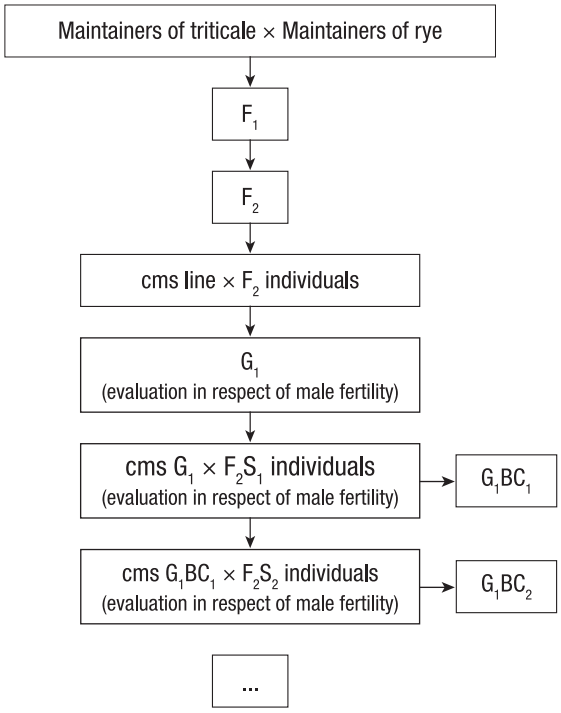

Figure 1. A diagram of procedures for developing G_1 and crossing with F_2 (maintainers of triticale × maintainers of rye) and inbred progenies of F_2

(Applied Biosystems). ISSR-PCR products were separated in a 1% agarose gel in TBE buffer. A DNA marker of 100 to 1000 bp and a concentration of 0.5 mg mL^{-1} (Fermentas) were used to determine the length of the ISSR-PCR fragments. The gels were imaged using ImageMaster VDS gel reader (Amersham - Pharmacia Biotech). Gel analysis was performed using GelScan ver. 1.45 (Kucharczyk - Electrophoretic Techniques), the software enables precise determination of PCR fragments. With the application of the software it is possible to detect differences with the accuracy of ten nucleotides.

F_1 hybrid plants were self-pollinated to obtain the F_2 generation. One hundred eighty F_2 hybrid individuals were tested for the presence of non-restoring genes. To do this, hybrid plants were used as pollinators in crosses to male sterile triticale lines: cms Salvo 15/1 and cms 19 (in BC_{17} and BC_{13} generations, respectively). G_1 progenies generated from these crosses were evaluated for male fertility. Subsequently, G_1BC_1 and G_1BC_2 backcross generations were obtained by using plants originating from fully male sterile progenies of the preceding generation (G_1 and G_1BC_1) as females, and inbred progenies of F_2 individuals (F_2S_1 and F_2S_2) as males. Male fertility of each generation was evaluated by examining seed set in heads, covered with isolation bags prior flowering, of 10-20 plants grown at wide spacing (20 × 40 cm) at the Experimental Station in Prusy, Poland near Krakow. Plants were considered male sterile if no seeds were recovered. The total procedure of crosses is presented in Fig. 1.

Results

In our study, only ten putative hybrid grains (0.14%) were obtained after pollinating over seven thousand flowers (Table 1). One hybrid was phenotypically similar to a male rye line and sterile, while the remaining hybrids, similar to female triticale lines, exhibited full male fertility and were used to generate the F_2 generation through selfing.

The hybrid nature of the F_1 plants from crosses of triticale and rye was confirmed using the ISSR01, ISSR02, ISSR06, ISSR07 primers. Our primers produced 24 to 49 amplification fragments of different lengths. Among them, several fragments were monomorphic, *i.e.* common to maintainers of triticale, maintainers of rye and their F_1 hybrids (Table 2). In most primer-genotype combinations, one to four fragments were specific to the rye maintainers and the F_1 hybrids. These data provide evidence for the hybrid character of the F_1 plants (Table 3, Suppl. Fig. S1 [pdf online]). These fragments were not observed in female triticale (19 and Salvo maintainers). In all F_1 hybrids, PCR fragments were detected that were absent from both parents (*e.g.* a 510 bp fragment generated with the ISSR01 primer in the hybrid derived from triticale and rye maintainers (19 × 585/92-1-2; Suppl. Fig. S1 [pdf online]).

In the set of hybrids, we obtained seven fragments using ISSR01 primer that were present in the male parents and the hybrids, and seven fragments that were present in the females and the hybrids. The ISSR03 primer generated the largest number of fragments, confirming the hybrid nature of the obtained hybrids. We identified 7 fragments that were present in the male parents and the hybrids, and 16 fragments that were present in the female parents and the hybrids (Table 3). This primer also proved to be the most useful for hybrid identification, since it enabled amplifying marker fragments for all analyzed genotypes. For example, three fragments (590, 420, 260 bp) produced with the ISSR01 primer were common to the rye maintainers,

Table 2. PCR fragment numbers obtained in all studied genotypes (triticale lines, rye lines, F_1 hybrids)

Primer	Total number of fragments per primer	Mean number of PCR fragments per plant	Range of PCR fragments per plant	Total number of fragments confirming hybrid nature of F_1 plants	Common fragments (bp)
ISSR01	42	10	8-12	14	730, 440, 180
ISSR03	49	13	10-15	23	860, 650, 360, 200
ISSR06	26	9	8-11	9	230, 200, 140
ISSR07	24	9	4-13	14	340, 300, 250

Table 3. Fragments observed in F_1 hybrids and their parental lines (female triticale lines and male rye lines) generated with ISSR primers (length of fragments in bp)

Primer	Common fragment	Hybrids			
		F_1: 19, S_{13} × 585/92-1-2, S_9	**F_1: 19, S_{13} × 585/92-6-1, S_9**	**F_1: 19, S_{13} × 541-6, S_{24}**	**F_1: Salvo × 541-6, S_{24}**
ISSR01	Male and hybrid	590, 420, 260	300, 240	300, 260	None
	Female and hybrid	810, 310	780, 620	730, 440, 390	None
ISSR03	Male and hybrid	230	710, 330, 260	880	680, 500
	Female and hybrid	580, 520, 480, 430, 390	860, 650, 520, 480, 390	650, 480, 360, 240	550, 390
ISSR06	Male and hybrid	470	300	None	620, 260
	Female and hybrid	360, 260	260, 200	None	690
ISSR07	Male and hybrid	450, 320, 210, 190	210,190	220	None
	Female and hybrid	470, 400, 270	470, 270	660, 270	None

used as males (585/92-1-2, S_9) and their F_1 hybrids (19 × 585/92-1-2, S_9), proving the hybrid origin of the F_1 plants (Table 3, Suppl. Fig. S1 [pdf online]). Two fragments (810, 310 bp) generated by the ISSR01 primer were common to triticale maintainers used as females (line 19) and their F_1 hybrids (19 × 585/92-1-2, S_9), proving the hybrid origin of the F_1 (Table 3, Suppl. Fig. S1 [pdf online]). All primer-genotype combinations are presented in Table 3, which is based on Suppl. Fig. S1 [pdf online]. Our research shows that in the case of the 19 S_{13} × 585/92-1-2, S_9 and the 19 S_{13} × 585/92-6-1, S_9 hybrids, it was possible to confirm the hybrid nature of the F_1 plants with all primers.

Male fertility of the 180 G_1, obtained from crosses of male sterile lines of triticale to individuals of the F_2 generation, measured by seed set, ranged from 0 to 37 grains per head. Seed sets within offspring varied from 0 and 10 seeds per head. Most progenies formed 0 to 2 per head (Fig. 2). Among the 180 G_1 progenies, 71 offspring (39.4%) were completely male sterile

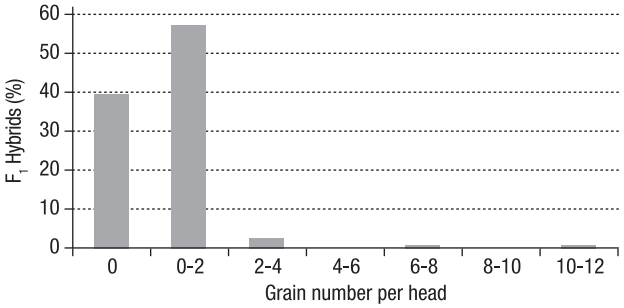

Figure 2. Seed set on isolated heads of F_1 hybrids (n = 180)

(Table 4). In subsequent generations (G_1BC_1 and G_1BC_2), obtained by pollination of completely male sterile plants from G_1 and G_1BC_1 offspring of F_2S_1 and F_2S_2 individuals (that were, in turn, generated by inbreeding of F_2 segregants), the proportion of male sterile individuals increased as a result of increasing homozygosity of males and selection of completely male sterile females. Fourteen F_2 segregants, as well

Table 4. Male sterile offspring (%) in consecutive generations of crosses of male sterile triticale lines to F_2 segregants

Generation	No. of progenies	(%)
G_1	180	39.4
G_1BC_1	104	65.4
G_1BC_2	44	75.0

as F_2S_1 and F_2S_2 individuals obtained from the F_2 plants (7.8%), exhibited consistent male sterility in G_1, G_1BC_1, and G_1BC_2 generations.

Discussion

At present only on a limited scale cytoplasmic male sterility is utilized to produce hybrid seeds in triticale (Nalepa, 1990; Góral, 2002; Ammar *et al*., 2006; Longin *et al*., 2012) because of limited number of restorer and maintainer lines in applied sterility systems based on *T. timopheevi* and *Ae. sharonensis* cytoplasms. The maintenance of male sterility in the *Ae. sharonensis* system is not problematic, since only 1-2% of cultivars restore male fertility in F_1 hybrids (Nalepa, 2003). Another system, cms-*T. timopheevi* is characterised by a low frequency of maintainers (Warzecha *et al*., 1998; Góral, 2002; Góral & Spiss, 2005; Góral *et al*., 2007).

Additionally, male sterility is not stable in different environments (Nalepa, 2003; Góral *et al*., 2006). Most strains and cultivars largely, albeit not fully, restore fertility of the *T. timopheevi* cytoplasm (Góral, 2002; Góral & Spiss, 2005; Góral *et al*., 2007). A frequency of over a 19% and 14% of maintainers and restorers respectively, for the cms-*T. timopheevi* system was detected in Mexican germplasm of spring triticale (Ammar *et al*., 2006). Because of the low frequency of maintainers in Polish germplasm, a great number of cultivars and lines must be screened, and new germplasm must be intentionally generated. New maintainers were obtained from the F_1 hybrids (non-restoring line × cultivar/strain) as a result of transferring maintaining genes to lines and cultivars from existing maintainers through recombination and homozygotic process with the application of a doubled haploid (DH) line technique. Over 9% of the DH lines generated that way sustained male sterility, and 1.5% fully restored fertility (Góral *et al*., 2007). In this study, we present an effort to implement non-restoring genes from rye to triticale through recombination and examination of their function.

In triticale breeding, genetic variation among existing breeding germplasm, as well as variation introduced from its progenitors, *i.e.* wheat and rye, can be utilized through genomic, chromosomal or gene recombination. In the present study, crosses conducted among maintainers of triticale and rye showed low efficiency. This contrasts with studies of other authors (Tarkowski & Otłowska, 1968; Guedes-Pinto *et al*., 2001; Hills *et al*., 2007), who obtained higher seed set, although crossability in these studies depended on the parental genotype and environmental conditions (Guedes-Pinto *et al*., 2001).

In the case of wide crossing, it is crucial to confirm the hybrid nature of the obtained plants. The hybrid nature can be verified based on morphological features revealed during the vegetative phase (Naresh *et al*., 2009) and after the transition to the generative phase (Ladizinsky, 2000). However, techniques allowing identification of hybrids in the early stages of plant development, for example in the seeds or seedlings stage, offer a huge advantage and provide the opportunity to eliminate very early the plants produced through self-pollination.

Molecular markers are frequently used for the identification of hybrid forms. The ISSR primers utilized in this work allowed the determination of the PCR profile specific to the parental forms and their segregation in the studied hybrids (Table 4, Suppl. Fig. S1 [pdf online]).

Grądzielewska *et al*. (2012) identified the presence of *Aegilops juvenalis* genetic material in seven triticale breeding lines using the ISSR-PCR method. Using 14 ISSR primers, they generated 240 DNA fragments, of which 72 (55%) were polymorphic. In our study, 4 primers generated 141 ISSR fragments, and 62 of them (43%) were polymorphic. Grądzielewska *et al*. (2012) reported that a single oligonucleotide generated 3 to 9 amplification fragments unique to hybrids, and one of the parent. Thirteen primers amplified *Ae. juvenalis* specific fragments that were also present in the hybrids.

In addition to the fragments from parents in the genomes of hybrids, unique fragments were reported missing in any parental forms. Similar results were observed by Feldman *et al*. (1997, 2012) in his research on hybrid wheat. He stated that in hybrids, especially those originated from wide crosses, there is a rearrangement of genomes consisting largely of deletions, methylation and gene silencing. Research by Feldman *et al*. (1997, 2012) has shown that deletion primarily undergo non-coding DNA regions including satellite,

but also to coding regions. Moreover, he observed that this phenomenon occurs particularly in the F_1 generation. A similar phenomenon was described by Han *et al*. (2005), who also analyzed wheat hybrids. ISSR markers utilized in this study allowed us to observe this phenomenon in triticale - rye hybrids. According to the above-cited authors, this phenomenon is associated with stabilization of hybrid genomes at the DNA level necessary for establishing such homeology, which enables the correct conjugations of chromosomes in meiosis.

The maintaining ability of F_2 recombinants and the F_2-derived lines was tested in crosses to male sterile lines of triticale with *T. timopheevi* cytoplasm. Among 180 progenies of these test crosses, 71 (39.4%) were completely male sterile and 14 F_2 segregants (7.8%) as well as their descendant, consistently sustained male sterility in G_1, G_1BC_1 and G_1BC_2 generations. These results suggest a possibility of obtaining new non-restorers lines for triticale of the *T. timopheevi* cms system through recombination of triticale and rye maintainers representing two distinct male sterility systems. Consequently, a more stable cms system, compared to the existing one, could be developed in triticale based on the *T. timopheevi* cytoplasm. The results do not prove directly that new maintainers possess non-restoring genes originating from rye genome, but they suggest such a possibility.

Acknowledgements

The research was supported by the Polish Ministry of Science and Higher Education - Contract No. DS/3129/KHRiN.

References

Ammar K, Crossa J, Pfeiffer WH, 2006. Developing a hybrid seed production system and evaluation of heterosis levels in hybrids from CIMMYT's spring triticale germplasm. Proc. of the 6th Int. Triticale Symp. Stellenbosch, South Africa, 3-7 September, pp: 65-67.

Bianco CL, Juan A, Fernández JA, Migliaro D, Crino P, Catalina Egea-Gilabert, 2011. Identification of F_1 hybrids of artichoke by ISSR markers and morphological analysis. Mol Breeding 27: 157-170.

Carvalho A, Matos M, Lima-Brito J, Guedes-Pinto H, Benito C, 2005. DNA fingerprint of F1 interspecific hybrids from *Triticeae* tribe using ISSRs. Euphytica 143: 93-99.

Curtis CA, Lukaszewski AJ, 1993. Localization of genes in rye that restore male fertility to hexaploid wheat with *T. timopheevi* cytoplasm. Plant Breed 111: 106-112.

Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM, 1997. Rapid elimination of low- copy DNA sequences in polyploid wheat: A possible mechanism for differentiation of homoeologous chromosomes. Genetics 147: 1381-1387.

Feldman M, Levy AA, Fahima T, Korol A, 2012. Genomic asymmetry in allopolyploid plants: wheat as a model. J Exp Bot 63: 5045-5059.

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

Góral H, 2002. Production of triticale (×*Triticosecale* Wittm.) hybrid seeds using the sterilizing cytoplasm of *Triticum timopheevi*. Cereal Res Com 30: 31-38.

Góral H, Spiss L, 2005. Hodowla dopełniaczy i restorerów dla systemu cms-*T. timopheevi* u pszenżyta jarego. Bull IHAR 236: 99-104.

Góral H, Warzecha T, Stojałowski S, Pojmaj M, Kurleto D, Trąbka A, Spiss L, 2006. Stability of male sterility and fertility restoration in the cms-*T. timopheevi* system in triticale. Folia Univ Agric Stetin 247 (100): 55-62.

Góral H, Pojmaj MS, Pojmaj R, 2007. Frekwencja genotypów dopełniających i restorujących dla systemu cms-*T. timopheevi* u pszenżyta ozimego. Bull IHAR 244: 155-160.

Grądzielewska A, Gruszecka D, Lesniowska-Nowak J, Paczos-Grzęda E, 2012. Identification of hybrids between triticale and *Aegilops juvenalis* (Thell.) Eig. and determination of genetic similarity with ISSRs. Genet Mol Res 11(3): 2147-2155.

Guedes-Pinto H, Lima-Britto J, Ribeiri-Carvalho C, Gustafson JP, 2001. Genetic control of crossability of triticale and rye. Plant Beeding 120: 27-31.

Han F, Fedak G, Guo W, Bao Liu B, 2005. Rapid and repeatable elimination of a parental genome-specific DNA repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. Genetics 170: 1239-1245.

Hills MJ, Hall LM, Messenger DF, Graf RJ, Beres BL, Eudes F, 2007. Evaluation of crossability between triticale (×*Triticosecale* Wittmack) and common wheat, durum wheat and rye. Environ Biosafety Res 6(4): 249-257.

Ladizinsky G, 2000. A synthetic hexaploid (2n = 42) oat from the cross of *Avena strigosa* (2n = 14) and domesticated *A. magna* (2n = 28). Euphytica 116: 231-235.

Liu LW, Wang Y, Gong YQ, Zhao YM, Liu G, Li XY, Yu FM, 2007. Assessment of genetic purity of tomato (*Lycopersicon esculentum* L.) hybrids using molecular markers. Sci Hortic 115: 7-12.

Longin CFH, Mühleisen J, Maurer HP, Zhang H, Gowda M, Reif JCh, 2012. Hybrid breeding in autogamous cereals. Theor Appl Genet 125: 1087-1096.

Nagaoka T, Ogihara Y, 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet 94: 597-602.

Nalepa S, 1990. Hybrid triticale: present and future. Proc 2nd Int Triticale Symp Passo Fundo, Rio Grande do Sul, Brasil, 1-5 October, CIMMYT, Mexico, pp: 402-407.

Nalepa S, 2003. Perspektywy hodowli pszenżyta w Resource Seeds Inc. w USA. Bull IHAR 230: 143-146.

Naresh V, Yamini KN, Rajendrakumar P, Dinesh Kumar V, 2009. EST-SSR marker based assay for the genetic purity assessment of safflower hybrids. Euphytica 170: 347-353.

Stepansky A, Kovalski I, Perl-Treves R, 1999. Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. Plant Syst Evol 217: 313-333.

Tarkowski Cz, Otłowska D, 1968. Badania nad heksaploidalnym triticale i jego mieszańcami z żytem i pszenicą [Studies on hexaploid triticale and its hybrids with rye and wheat]. Hodowla Roslin (Plant Breeding) 5: 577-593.

Warzecha R, Salak-Warzecha K, Staszewski Z, 1998. Development and use of triticale CMS system in hybrid breeding. Proc 4th Int Triticale Symp Red Deer, Alberta, Canada, July 26-31, pp: 79-85.