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ORIGINAL PAPER

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Dominant male sterility in sorghum: effect of nuclear background on inheritance of tissue-culture-induced mutation

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Abstract Occurrence of genetic instability and formation of stable mutations are basic genetic processes. This study demonstrates that nuclear background may influence the formation of stable dominant nuclear gene of male sterility (MS) on the basis of unstable mutation, which was induced in tissue culture of the sorghum haploid (cv. Milo-145). The mutants with complete or partial MS segregated in variable ratios in the progenies of diploid regenerants were obtained from different experiments on cultivation of haploid tissues. In the Milo-145 genetic background the mutation demonstrated somatic instability and was gradually eliminated by self-pollination of partially sterile plants. Hybridization of the MS-plants with the sorghum line SK-723, a fertility-restorer of the cytoplasmic MS A1 (*milo*) type, maintained the induced mutation. By repeated backcrossing of MS-plants with SK-723, the male-sterile versions of this line (SK-723- Ms_{tc}) have been created. In BC-generations, fertile, partially and completely sterile plants were observed. The MS-plants from BC-generations are proposed to contain a dominant gene Ms_{tc} while fertile plants were ms_{tc}/ ms_{tc} homozygotes. Crossing the original MS-plants with SK-723 was a key factor in stabilization of the Ms_{tc} gene. Dominant expression of the Ms_{tc} was observed in male-sterile versions of other sorghum lines created by backcrossing to SK-723- Ms_{tc}. The lines fertility-restorers for this mutation have been revealed. In the crosses of restored F_1 hybrids with emasculated plants of the non-restoring line, the Ms_{tc} has been transferred through the pollen and manifested in the F_1 generation. The possibility of the Ms_{tc} originating as a result of interaction of an unstable allele of the Milo-145 with the SK-723 genome is discussed.

Keywords Sorghum · Male sterility · Nuclear–cytoplasmic interactions · Somaclonal variation · Genetic instability

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Introduction

Induction of genetic variation and maintenance of induced mutations are key genetic processes. In addition to different environmental factors, a number of genetic systems inducing nuclear or cytoplasmic mutations have been identified. Among them are specific gene-mutators that cause genetic instability in the nuclear genome (McClintock 1965; Yu et al. 2000; Lisch 2002), in the plastome (Kirk and Tilney-Bassett 1978; Chang et al. 1996) and mutations in the mitochondrion (He et al. 1995; Veprev et al. 1997; Kuzmin et al. 2005). Rearrangement in the mitochondrial genome leading to cytoplasmic male sterility (CMS) was shown to be induced by remote hybridization (Ogihara et al. 1999). Similarly, the increased rate of nuclear gene mutations in alien cytoplasms in wheat (Maan 1979) and heritable inactivation of dominant genes in homozygous maize lines obtained by transferring haploid nuclear genomes into alien cytoplasm by androgenesis (Zavalishina and Tyrnov 1998) have been described. However, little is known about genetic systems influencing maintenance of induced genetic variation, although, genetic background may offer significant effect on stability of induced mutation both in the nuclear and cytoplasmic genomes.

Effective tool for inducing genetic instability in the nuclear and cytoplasmic genomes is tissue culture. Plant regeneration from cells of differentiated tissues breaks normal epigenetic mechanisms involved in genetic control of plant development and this induces a number of phenomena of genetic instability such as transposon activation and gene silencing (Peschke and Phillips 1992; Kaeppler et al. 2000). In certain cases,

these unstable genetic events may be fixed and inherited as stable gene mutations or eliminated in the test-cross progenies.

The MS mutations are convenient models for studying these phenomena because anther and pollen development are complex processes that are controlled by closely interacting nuclear and cytoplasmic genes. In the case of CMS, male-sterile phenotype occurs as a result of the functioning of aberrant mitochondrial CMS-inducing genes that manifests only in the absence of dominant nuclear fertility restorer genes. These genes encode specific proteins suppressing expression of CMS-inducing genes at the post-transcriptional level (reviewed in: Hanson and Bentolila 2004). High variability of mitochondrial genome in tissue culture conditions gives opportunity for creating new genotypes with CMS (reviewed in: Elkonin and Tyrnov 2001). The CMS mutations are maintained by specific lines sterility-maintainers with recessive alleles of fertility restorer genes. Contrary to CMS, nuclear male sterility (NMS) is caused by mutations of nuclear genes involved in the control of anther and pollen development. The NMS mutations express in different cytoplasms and do not need special lines sterility-maintainers for their maintenance.

Previously, diploid plants with MS have been regenerated from tissue culture of haploid sorghum, *Sorghum bicolor* (Elkonin et al. 1993). The mutants demonstrated somatic segregation of genetic factors controlling MS. In addition, they showed non-Mendelian segregation of sterile, partially sterile, and fertile plants in the progeny of partially sterile plants and in crosses with line SK-723, which is a fertility-restorer of the A1 cytoplasm of sorghum. In some hybrid combinations partial or complete restoration of male fertility was observed. On the basis of these data, the induced mutation was proposed to be cytoplasmic (Elkonin et al. 1994). However, further investigations of BC-hybrids with the line SK-723 reported in this paper have revealed the presence of a dominant nuclear gene for MS that originated, probably, as a result of specific interaction of the SK-723 nuclear genome with unstable mutation induced in tissue culture.

Materials and methods

Male-sterile mutants were originally observed among the diploid plants and their progeny regenerated from calli derived from leaves of sorghum haploid of cv. Milo-145 [*Sorghum bicolor* (L.) Moench subsp. *bicolor* race *durra*] (Elkonin et al. 1993). This line had CMS-inducing cytoplasm (milo, A1) and fertility restorer genes. The mutants 47-86 and 187-85, and the mutant G3 were derived from different tissue culture experiments. The mutants from different regenerant families were characterized by small and dry anthers that did not shed pollen after panicle shaking and did not set seed or had a poor seed set under isolation. Under open-pollination conditions they had normal seed set. In this research, before conducting a test of allelism, the male-sterile mutants from different regenerant families (denoted by their specific numbers) are assumed to contain one and the same mutation since they demonstrated similar inheritance pattern of sterility.

Table 1 Fertile sorghum lines used in test-crosses with male-sterile mutants induced in tissue culture

Name of the line	Race	Origin	Reaction to CMS type[a]			
			A1	A2	A3	A4
SK-723	Caudatum	Selection from the cross Negrityanskoye k-3366/Sudangrass Krasnoplenchataya-16/1E	R	PR	B	B[b]
Milo-145	Durra	Collection line	R	Nd	B	Nd
Milo-10	Durra	Collection line	R	PR	B	B
Feterita-14	Caudatum	Selection from progeny of spontaneous early-maturing mutant from Feterita k-142	R	R	B	R
Yefremovskoye-2	Kafir	Collection line	B	B	B	B
Saratovskoye-3	Bicolor	Collection line	B	B	B	B
Volzhskoye-2	Kafir	Selection from Norghum-165	R	R	B	Nd
Volzhskoye-4	Kafir	Selection from the cross of induced mutants from Norghum-165 and Yefremovskoye-2	R	R	B	Nd
Volzhskoye-4w	Kafir	Selection from the cross Volzhskoye-4/Gvineiskoye k-2730	R	R	B	Nd
Pishchevoye-614	Durra	Selection from the cross Gvineiskoye k-2730/Milo-10	R	R	B	B
Belenkiy	Caudatum-kafir	Hegari k-241, selection from the progeny of free-pollinated panicle	R	R	B	Nd
Ksyusha	Durra-caudatum	Selection from the cross Palestinskoye-35/Solar-01	R	PR	B	Nd
KVV-52	Durra-kafir	Selection from the cross Rosinka-2 (*S. bicolor* × sudangrass) /Norghum-165	R	B	B	B
KVV-181	Caudatum	Selection from the cross Rosinka-2/Feterita k-2312	R	B	B	B[b]
KVV-114	Kafir-bicolor	Selection from hybrid population KVV-97 (*S. bicolor* × sudangrass)/Soriz	R	B	B	B
KVV-86	Kafir-bicolor	Selection from hybrid population *S. bicolor* × sudangrass	R	B	B	B

[a] *R* fertility-restorer, *PR* partial restorer, *B* sterility-maintainer, *nd* not determined
[b] Segregated fertile plants in BC-generations

For genetic analysis of the induced mutation, the mutants from different regenerant families have been crossed to a number of lines, fertility-restorers, and sterility-maintainers for CMS-inducing cytoplasms A1–A4 (Table 1). These tester lines are early-maturing selections from the hybrid populations; they were taken from collection of the All-Russian Institute for sorghum and maize, Saratov, Russia. Fertility reactions of these lines were previously studied in test-crosses with series of alloplasmic isonuclear lines having T×398 genome that was provided by Dr. K.F. Schertz (Texas Agricultural Experimental Station, USA).

For crossing, the bagged panicles of the mutants were carefully expected in 2–3 days after anthesis at the panicle base. Those panicles which had no fertile anthers and pollen in the bags and having 'fresh' stigmas on the whole panicle were considered 'male sterile' and were then selected for crosses. F_1, F_2, and BC-generations as well as the progenies from selfed fertile segregates were grown in experimental fields of the Agricultural Research Institute for the South-East Region and of the All-Russian Institute for sorghum and maize (both located in Saratov, Russia). Fertility was determined by percentage seed set on panicles bagged before anthesis. The plants were classified as sterile (s, 0% or 1–2 single seeds), partially sterile (ps, 1–75%, usually 5–15%), or fertile (f, >75%). The ps-plants were characterized by sectors of fertile flowers on one or a few panicle branches usually located at the basal part of the panicle.

The χ^2 -test was used to determine the fit of observed segregation ratios of sterile and fertile plants to the expected segregation ratios. The partially (ps) and completely sterile (s) plants were bulked for χ^2 -test. The number of partially sterile and sterile plants in different regenerant progenies was compared using Fisher's *F*-test.

Results

Inheritance of male sterility by the mutant genome

Mutants with complete or partial MS were found among the primary regenerants (R_0) and in subsequent generations of self-pollinated fertile and partially sterile regenerants from different experiments on cultivation of sorghum haploid of cv. Milo-145. The ratio of fertile, partially and completely sterile plants varied considerably between families and between generations. Usually, self-pollination of fertile plants gave only fertile and partially sterile plants, while completely sterile plants were observed only in the progenies of partially sterile plants (Table 2). Partially sterile plants from the late generations yielded significantly fewer sterile and partially sterile plants. One and the same plant often produced a first panicle that was sterile and second panicle that was partially sterile. No sterile plants were observed in the progeny of the latter panicles. Pollination of sterile panicles by the original line, Milo-145, gave a ratio of fertile, and partially and completely sterile plants in the F_1 and subsequent generations that resembled segregation in the progeny of partially sterile plants (Table 3). This inheritance pattern could not be explained by segregation of either a dominant or recessive nuclear gene and was more likely due to segregation of cytoplasmic organelle sequences or gradual restoration of function of one or more silenced genes, which had been silenced in tissue culture.

As a similar inheritance pattern was found in the progeny of mutants from different regenerant families it was supposed that different regenerants contained one and the same mutation.

Table 2 Examples of segregation for male sterility in the progeny of some regenerants (R_0)

Regenerant no	Generation	Number of plants[a]		
		f	ps	s
G3	R_1	–	5	6
	R_2 (Selfed ps-plant no.1)	44	40	2
	R_2 (Selfed ps-plant no.2)	12	22	14
	R_2 (Selfed ps-plant no.3)	–	4	4
	R_2(Total from three ps-plants)	68	76	21
	R_3 (Total from two ps-plants)	23	19*	4*
	R_3 (Total from three f-plants)	104	26	–
4–36	R_1	3	7	4
	R_2 (Selfed f-plant no.1)	34	44	1
	R_2 (Selfed f-plant no.2)	6	11	2
	R_2 (Total from two selfed f-plants)	40	55	3
	R_2 (Selfed ps-plant no.1)	22	14	8
	R_2 (Selfed ps-plant no.2)	6	33	15
	R_2 (Selfed ps-plant no.3)	3	6	1
	R_2 (Total from three selfed ps-plants)	31	53	24
	R_3 (Total from seven ps-plants)	73	19*	1*
	R_3 (Total from four f-plants)	114	16	–
B18	R_1	–	4	4
	R_2 (Total from four ps-plants)	20	26	7
	R_3 (Total from five ps-plants)	70	26*	2*
	R_3 (Total from four f-plants)	155	27	1

[a] *f* fertile, *ps* partially sterile, *s* sterile plants
*differed significantly ($p < 0.05$) from the corresponding value in the previous generation

Inheritance of male sterility in testcrosses with standard fertility restorers

To compare the induced mutation with the known types of sorghum CMS-inducing cytoplasms, the male-sterile plants from different regenerant families (G3, 47-86, 187-85) were crossed to standard testers for fertility restoration and sterility maintenance of these cytoplasms (Table 3). The induced mutation was expressed in hybrids with both restorers and maintainers of the A1 and A2 CMS types of sorghum, but no progeny with strict maternal inheritance of MS was observed. These observations make it less probable that the induced mutation was cytoplasmic and show its difference from the A1 and A2

Table 3 Inheritance of induced mutations for male sterility in test-crosses with fertile sorghum lines

Tester line		Number of plants[a]		
		f	ps	s
Test-crosses with MS-plants of mutant G3 with Milo-145 genome				
SK-723		1	2	6
KVV-52		9	–	–
KVV-181		9	–	–
Milo-145	Mutant plant N1	4	8	6
	Mutant plant N2	10	7	1
KVV-114		15	–	–
Yefremovskoye-2		5	3	2
Volzhskoye-4		11	–	–
Test-crosses with MS-plants from BC_4 of the mutant 47-86 backcrossed to SK-723				
KVV-52		9	3	5
Pishchevoye-614		6	2	3
Belenkiy		9	2	6
KVV-86		21	8	9
Volzhskoye-4w		12	7	9
Milo-10		15	–	–
Volzhskoye-2		12	–	–
KVV-181		12	–	–
Feterita-14		11	–	–
Ksyusha		15	–	–
Saratovskoye-3		2	15	–

[a] *f* fertile, *ps* partially sterile, *s* sterile plants

CMS types. Phenotypic expression of this mutation, which creates degeneration of the sporogenous tissue of anthers and microspores and abnormalities of meiosis in non-degenerated microsporocytes (Tsvetova and Elkonin 2003), was also quite different from the gametophytic A3 and non-dehiscent A4 types of CMS. Among tested lines, SK-723 possessed the maximum ability to maintain the induced MS. Different sterile plants from different regenerant families yielded a similar inheritance pattern after pollination with SK-723: a significant preponderance of sterile and partially sterile plants in the F_1, and the appearance of fertile plants in BC-generations (Table 4, Fig. 1).

Dominant inheritance of male sterility

Male sterile F_1 hybrids with the line SK-723 from different regenerant families (187-85, 47-86, G3) were repeatedly backcrossed to SK-723. In backcross generations, plants with complete or partial restoration of male fertility appeared in all families (Table 4). Segregation of sterile and fertile plants in a majority of crosses fitted a 1:1 ratio but significant deviations were observed in a number of families, such as, preponderance of sterile plants in 57/90 (BC_3), 12/96 (BC_6), 12/97, 27/97, 106/02 (BC_7), 3/03 (BC_8), and appearance of non-segregating progenies completely lacking fertile plants (8/96, 11/96, 9/00, 10/00—all from the BC_6-generation). However, in the progeny of male-sterile plants from the non-segregating families, that is, in the next BC-generation, a normal segregation ratio was observed again.

Fertile plants from different backcross generations proved to be homozygous and did not segregate sterile offspring. In their sib-crosses with sterile siblings segregation for sterile and fertile plants corresponding to the ratio 1:1 was observed (Table 5). This result indicates that a nuclear dominant gene controls MS in these crosses. For its designation we used symbol Ms_{tc} (*male sterile from tissue culture*. In this case, male-fertile plants from backcross generations are homozygotes ms_{tc} /ms_{tc} and their sterile siblings are heterozygotes Ms_{tc} /ms_{tc}.

In agreement with this hypothesis, fertile plants in the progenies from sib-crosses should be also homozygotes ms_{tc} /ms_{tc} while male-sterile plants should be heterozygotes Ms_{tc} /ms_{tc}. Testcrosses of sterile and fertile plants from the sib-cross 34-10s/34-1f confirmed this hypothesis (Table 5).

It should be noted that segregation of partially sterile plants was observed sometimes in the progeny of some fertile siblings but completely sterile plants were always absent. Such partially sterile plants were observed also in some seasons in the recurrent parent, SK-723.

Analyses of the progeny of partially sterile plants segregating in BC-generations revealed that they were genetically heterogeneous. Sometimes, they produced entirely sterile progeny, but more often they segregated for fertile, partially sterile, and sterile plants (Table 5). The appearance of sterile plants in the self-pollinated progenies of some partially sterile plants as well as the mode of expression of partial sterility (sectors of fertile flowers or separate panicle branches) indicate that partial sterility does not result from the mutation of a gametophytically expressed gene. Segregation in some of the self-pollinated progenies of partially sterile plants fit to the ratio 1 fertile: 3 (sterile + partially sterile) that would appear if heterozygous plants Ms_{tc} /ms_{tc} spontaneously produce some fertile pollen having both Ms_{tc} and ms_{tc} genotypes. As a result of self-pollination by this pollen, in the next generation would appear homozygous sterile plants Ms_{tc} /Ms_{tc} that will produce non-segregating progeny being pollinated by the SK-723 pollen. Testcrosses of sterile plants from the progeny of ps-plant 106-1 (BC_7), which had small fertile branch at the panicle base, to the SK-723 showed that among four studied progenies, was found one non-segregating family that consisted of only sterile plants (s4/SK-723, Table 5). However, sometimes partially sterile plants produce almost completely fertile progeny (10–2 and 12–2ps from BC_7, Table 5). Being test-crossed to sterile siblings these partially sterile plants produced families segregating in the ratio 1:1. These data indicate that fertile sectors on panicles of these partially sterile plants may lack Ms_{tc} gene, perhaps, as a result of mutation $Ms_{tc} \rightarrow ms_{tc}$.

Table 4 Segregation for male sterility in families from different backcross generations obtained by crossing male-sterile sorghum mutants derived from tissue culture, with the line SK-723

Generation	Family	Year	Number of plants[a]			χ^2	*P*
			f	ps	s		
Mutant 187-85							
F_1	13/88	1988	4	–	20	–	–
BC_1	11/89	1989	2	2	19	–	–
BC_2	46/90	1990	15	1	17	0.273	0.75–0.50
Mutant 47-86							
BC_2	21/89	1989	2	1	6	–	–
BC_3	57/90	1990	1	–	16	–	–
		1991	–	3	34	–	–
BC_4	8/91	1991	15	–	11	0.615	0.50–0.25
BC_5	7/95	1995	9	1	14	1.500	0.25–0.10
	19/95	1995	12	2	18	2.000	0.25–0.10
BC_6	8/96	1996	–	1	23	–	–
		1999	–	–	11	–	–
	11/96	1996	–	–	20	–	–
	10/96	1996	13	1	17	0.806	0.50–0.25
	9/00	2000	–	4	21	–	
	10/00	2000	–	8	18	–	
	12/96	1996	4	2	15	8.048	< 0.05
BC_7(from 11/96)	27/97	1997	3	1	17	–	
(From 8/96)	18/97	1997	12	3	10	0.040	0.90–0.75
	12/97	1997	5	2	17	8.167	< 0.05
(From 9/00)	9/01	2001	14	–	9	1.087	0.50–0.25
	106/02	2002	2	1	10	–	
(From 10/00)	29/02	2002	9	1	11	0.429	0.75–0.50
BC_8 (From 106/02)	5/03	2003	11	1	10	0.0	> 0.95
	6/03	2003	12	9	2	0.043	0.90–0.75
(From 29/02)	3/03	2003	1	3	29	–	
BC_9 (From 3/03)	10/04	2004	12	–	11	0.043	0.90–0.75
	13/04	2004	9	2	11	0.727	0.50–0.25

[a] *f* fertile, *ps* partially sterile, *s* sterile plants; χ^2 was counted for 1f:1(ps + s) ratio

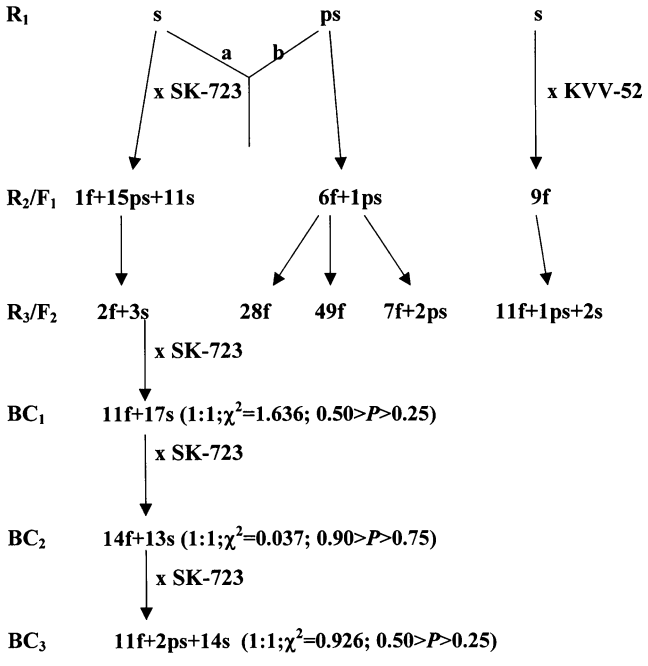

Fig. 1 Inheritance of male sterility in the progeny of two plants from the regenerant 'G3' family. a, b–sterile and partially sterile panicles of one and the same plant. *f* fertile, *ps* partially sterile, *s* sterile plants; figures mean the number of plants observed; R_1–R_3—self-pollinated progenies of original regenerant (R_0)

In the crosses of sterile plants from BC-generations with SK-723 with some other sorghum lines, including standard fertility restorers of the A1 cytoplasm, the Ms_{tc} gene expressed in the F_1 (e.g. in the testcrosses of the BC_2 47-86/SK-723, see Table 3). These data supported the hypothesis that the Ms_{tc} gene is not allelic to genes governing male fertility restoration in the A1 cytoplasm. After selecting male-sterile plants from these hybrid combinations and backcrossing them to the corresponding paternal line, MS was maintained in the backcross generations, with the ratio of sterile to fertile plants fitting a 1:1 ratio (Table 6). However, in some BC-generations with Volzhskoye-4w significant deficiency of sterile plants was observed, whereas in BC_8–BC_9 generations of the hybrids with Pishchevoye-614, non-segregating families lacking fertile plants were found.

Thus, by repeated backcrossing of MS-plants from BC-generations with SK-723 to the lines Pishchevoye-614, Volzhskoye-4w, and Belenkiy, the male-sterile versions of these lines with the Ms_{tc} gene have been created.

Analysis of the origin of the Ms_{tc} mutation

Of great importance for understanding genetic control of induced mutation has been the analyses of its origin. Above-stated data show that hybridization of the mu-

Table 5 Characterization of the progenies of fertile and partially sterile segregants from backcross generations of male-sterile mutants with the line SK-723, and their hybrids to sterile full sibs and testcross hybrids to SK-723

Progeny	Number of plants[a]			Ratio	χ^2	*P*
	f	ps	s			
Fertile and sterile full sibs from the BC_2 and BC_4 progenies of the mutant 47-86						
31-1s/31-2f (BC_2)	12	3	9	1f:1(ps + s)	0.0	> 0.95
31-2f	25	–	–			
142-10s/142-1f (BC_4)	18	3	19	1f:1(ps + s)	0.400	0.75–0.50
142-1f	7	–	–			
142-2f	34	–	–			
142-3f	78	–	–			
Fertile and sterile full sibs from the family 34/90 (BC_1 of the mutant 187-85)						
34-10s/34-1f	19	2	21	1f:1(ps + s)	0.381	0.75–0.50
34-1f	26	10	–			
34-13s/34-3f	15	2	20	1f:1(ps + s)	1.324	0.25–0.10
34-3f	30	8	–			
34-14s/34-4f	17	2	17	1f:1(ps + s)	0.111	0.75–0.50
34-4f	26	4	–			
34-15s/34-5f	11	5	22	1f:1(ps + s)	6.737	< 0.05
34-5f	46	5	–			
Fertile and sterile full sibs from the progeny 34-10s/34-1f						
s-1/f-2	10	5	9	1f:1(ps + s)	0.667	0.50–0.25
f-2	18	–	–			
s-3/f-4	5	1	13	1f:1(ps + s)	4.263	< 0.05
Examples of self-pollinated progenies of ps-plants from BC-families of the mutant 47-86						
34-1ps (BC_2)	6	1	16	1f:3(ps + s)	0.014	> 0.95
142-1ps (BC_4)	7	12	12	1f:3(ps + s)	0.097	0.90–0.75
8-1ps (BC_6)	4	7	7	1f:3(ps + s)	0.074	0.90–0.75
3-1ps (BC_8)	3	4	15			
142-2ps (BC_4)	–	8	2			
26-1ps (BC_2)	–	–	11			
106-1ps (BC_7)	–	–	8			
Test-crosses of sterile plants from the progeny of ps-plant (106-1ps, BC_7) to SK-723						
s-1/SK-723	7	2	7	1f:1(ps + s)	0.250	0.75–0.50
s-2/SK-723	14	2	7	1f:1(ps + s)	1.087	0.50–0.25
s-3/SK-723	8	5	6	1f:1(ps + s)	0.474	0.50–0.25
s-4/SK-723	–	–	19			
Partially sterile and sterile full sibs from BC_7 families of the mutant 47-86						
10-1s/10-2ps	10	–	7	1f : 1s	0.529	0.50–0.25
10-2ps	10	–	–			
12-1s/12-2ps	13	–	8	1f : 1s	1.190	0.50–0.25
12-2ps	18	1	–			

[a] *f* fertile, *ps* partially sterile, *s* sterile plants

tants with the line SK-723 allowed the maintenance of induced MS.

A key role for the SK-723 genome in the occurrence of the Ms_{tc} mutation was revealed from analyses of the following data (Fig. 1). In one of the plants from the R_1 generation (G3 family) the first panicle was sterile, while the second was partially sterile. The sterile panicle was crossed to SK-723. In the F_1 of this cross, sterile and partially sterile plants predominated. Subsequent backcrossing of sterile plants with SK-723 showed a typical dominant inheritance of MS, as in other mutants, which were backcrossed with SK-723 (Table 4). Remarkably, in the self-pollinated progeny of the second panicle of the same plant, R_2, a few fertile and one partially sterile plants were observed; sterile plants were also absent in the R_3 under self-pollination of both fertile and partially sterile plants from R_2. Thus, in the absence of the SK-723 genome, the mutation to MS was eliminated.

Another sterile plant from this family pollinated by the line KVV-52 produced fertile F_1 hybrids. However, when this line was crossed with a sterile plant from a BC-family with SK-723, it produced both sterile and fertile F_1 hybrids (Table 3). These results clearly demonstrate that the original male-sterile mutants obtained from tissue culture of Milo-145 lacked a dominant gene for MS.

Ms_{tc} transfer through the pollen

For further investigating genetic nature of induced mutation we undertook crosses to study transmission of genetic factor(s) inducing MS through the pollen. For this purpose, emasculated plants of the line Belenkiy, which is a maintainer for the Ms_{tc}-induced MS (Table 6), were crossed with restored F_1 hybrids SK-723(Ms_{tc} /ms_{tc})/KVV-181 that expressed normal male fertility. In the case of restoration of male fertility either by non-allelic fertility restoring gene (Rf_{tc}) or by over-dominant fertility-restoring allele (Ms_f) in the ms_{tc}-locus

Table 6 Expression of the Ms_{tc} gene in BC-hybrids with different sorghum lines

Recurrent parent	Generation	Number of plants[a]			χ^2	P
		f	ps	s		
Pishchevoye-614	F_1	3	–	4	–	
	BC_1	17	1	9	3.240	0.10–0.05
	BC_2	18	5	14	0.027	0.90–0.75
	BC_5	13	3	15	0.805	0.50–0.25
	BC_8	12	–	11	0.043	0.90–0.75
		–	–	18	–	
		1	1	14	–	
	BC_9[b]	10	5	5	0.0	> 0.95
		–	5	10	–	
Volzhskoye-4w	F_1	12	7	9	0.571	0.50–0.25
	BC_1	14	6	6	0.154	0.75–0.50
	BC_2	9	2	7	0.0	> 0.95
	BC_3	3	–	4	–	
		11	3	–	–	
	BC_4	30	5	46	5.444	< 0.05
	BC_5	26	2	2	–	
		12	5	5	0.182	0.75–0.50
	BC_6	11	–	9	0.200	0.75–0.50
Belenkiy	F_1	9	2	6	0.071	0.25–0.10
	BC_1	17	8	17	1.524	0.25–0.10
	BC_2	14	2	15	0.290	0.75–0.50
	BC_3	28	–	31	0.153	0.75–0.50

[a] *f* fertile, *ps* partially sterile, *s* sterile plants; χ^2 was counted for 1f:1(ps + s) ratio
[b] from non-segregating BC_8 family

that can suppress expression of the Ms_{tc}, the pollen of restored F_1 hybrids should contain the Ms_{tc} gene. Indeed, the analysis of two testcross hybrids revealed nine fertile and five sterile plants in one family, and 14 fertile and ten sterile plants in another family. This fact confirmed the presence of the dominant gene for MS in the pollen of restored F_1 hybrids and the nuclear location of genetic factor(s) conditioning this type of MS.

Discussion

The data presented above on inheritance of MS mutation induced in tissue culture of sorghum haploid (cv. Milo-145) demonstrates that substitution of the nuclear genome of the mutants by genome of the line SK-723 allowed the maintenance of induced mutation. This result was observed in three different mutants obtained in two different experiments (47-86 and 187-85 in one experiment, and G3 in another) on cultivation of somatic tissues (leaves) of the haploid and, therefore, represents a regular phenomenon. These data point on the specific interaction of genomes of Milo-145 mutants and SK-723. The mechanism of such interaction remains unclear but it is evident that under the influence of the SK-723 genome an unstable mutation induced in tissue culture was converted into a stable dominant gene, or the unstable MS-inducing gene might have pre-existed in the Milo-145 genome, its expression could be activated by tissue culture, and, further on, it became more stable in the nuclear-substituted lines with SK-723 genome. Such activation of unstable genetic events, including paramutation and transposons, and induction of gene silencing, are well-known phenomena in tissue culture conditions (Peschke and Phillips 1992; Kaeppler et al. 2000). Perhaps specific ability of SK-723 to maintain tissue culture-induced MS may be involved with the presence of any genetic factor(s), conditioning elevated level of abnormalities in microsporogenesis in this line and its partial MS in some seasons (Tsvetova and Elkonin 2003). Perhaps, formation of relatively stable gene inducing complete MS may result from interaction of these genetic factor(s) with genetic factor(s) of Milo-145 activated by tissue culture conditions.

It should be noted that original mutants had a number of features characteristic of cytoplasmic mutations (somatic segregation of sterility factors, predominantly maternal inheritance of MS in the F_1–BC_1 generations and in some late backcross generations). Such a maternal inheritance of MS in hybrids of Milo-145, which had sterility-inducing *milo* cytoplasm and fertility restorer genes for this cytoplasm, and SK-723, which is also fertility-restorer for the *milo* cytoplasm, might be observed if the original mutation was cytoplasmic but differed from the *milo* cytoplasm. In this case, the appearance of homozygous fertile plants in BC-generations could be explained either as a consequence of cytoplasmic instability or as a result of segregation of recessive fertility restorer genes. Such interaction of dominant sterility-maintaining nuclear genes and cytoplasm is known to control CMS in carrot (Timin and Dobrutskaya 1981), orchardgrass (Filion and Christie 1966) and *Solanum* (Grun 1976). We observed the dominance of nuclear stertility-maintaining genes of some cultivars in the A4 and '9E' cytoplasms of sorghum (Elkonin et al. 1998). However, the transfer of genetic factor(s) of induced MS through the pollen that has been demonstrated in this study, excludes this supposition.

An interesting phenomenon is formation of non-segregating progenies completely or almost completely lacking fertile plants in the late BC-generations, which were observed in the lines with both SK-723 and Pishchevoye-614 nuclear background. Such a maternal inheritance of MS is a characteristic feature of CMS mutations. However, in our mutants no stable maternal inheritance of MS was observed: in the progeny of male-sterile plants from the non-segregating families, that is, in the next BC-generation, a normal segregation ratio was found that turns down hypothesis on CMS mutation. These deviations could not be explained as maternally inherited epigenetic changes induced by tissue culture because they were observed in late BC-generations (starting from BC_6) while BC_4 and BC_5 had normal segregation ratio (1:1). The families, which were characterized by a disturbed segregation ratio, manifested this disturbance in different seasons suggesting that these deviations were not caused by action of environmental factors of a given season. The formation of non-segregating progenies did not depend on genotype

of a given paternal plant used to obtain next BC-generations, and therefore, was not due to heterozygosity of recurrent lines, SK-723 and Pishchevoye-614 (data not shown here). A possible explanation is that maternal plants used to obtain non-segregating families may be Ms_{tc} /Ms_{tc} homozygotes. Such homozygotes could appear among BC-progenies if the maternal plants (Ms_{tc} / ms_{tc}), which were used for crossing with recurrent line, were indeed partially sterile and produce Ms_{tc} /Ms_{tc} homozygotes as a result of self-pollination. In the next BC-generation these Ms_{tc} /Ms_{tc} homozygotes will produce only sterile plants (non-segregating progenies) in crosses with recurrent line. Perhaps, the distorted segregation ratios in some BC-generations with Volzhskoye-4w (Table 6) may be also explained by partial self-pollination of maternal plants, which were taken into crosses.

Remarkably, induced MS is expressed as a dominant mutation only in some genotypes. The F_1 hybrids with a number of other lines had normal pollen fertility and, therefore, these lines are fertility-restorers for this type of MS, while the former ones are sterility-maintainers. Similar specific types of MS, that manifests in some genotypes as a dominant mutation, while in others its expression is suppressed, have been revealed in millet (Hu et al. 1986), rice (Yan et al. 1989), and rape (Zhou and Bai 1994). It should be also noted that similar expression of MS was characteristic for spontaneous mutation, which was revealed in male-sterile plant of 'Day' variety of sorghum (Stephens et al. 1952). This fact points on parallelism of tissue culture-induced and spontaneously occurring genetic variation. The allelic relationships of these mutations would be ascertained in future.

Summarizing, for the first time in sorghum we have described an induced dominant mutation conferring MS. To our knowledge, this is the first report of experimentally obtained dominant MS in higher plants. According to the extensive review of Kaul (1988), all previously reported dominant mutations to MS have been spontaneous mutants. The Ms_{tc} mutation may be used in practice for developing sorghum hybrid populations maintaining heterosis effects for several generations owing to cross-pollination of sterile and fertile plants.

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