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*Original scientific paper***ONTOGENETICALLY-REGULATED MALE STERILITY IN TISSUE CULTURE - INDUCED AND SPONTANEOUS SORGHUM MUTANTS**

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Variability of male fertility expression in the AS-1 line, a somaclonal variant obtained from tissue culture of CMS-plant, and in the progeny of revertant '124-1' obtained from fertile tiller, which developed on CMS-plant transferred from the field to the greenhouse, was investigated. Both revertants were characterized by similar expression of male fertility during plant ontogenesis: the panicle on the main tiller was almost completely sterile whereas formation of fertile pollen grains and seed set were observed on the panicles of the shoot tillers. A clear basipetal gradient of male fertility was manifested on all panicles: the base had significantly higher per cent of fertile pollen grains in comparison with the middle part, while in the top the anthers were either absent or had few sterile pollen grains. Such an ontogenetically-regulated restoration of male fertility was controlled by nuclear genes and could be transferred through the pollen in crosses with progenitor CMS-line. Growing of AS-1 plants in the growth chambers simultaneously under a long (16/8) and a short (12/12) daylength conditions demonstrated that differences of fertility level in different tillers was not caused by change of photoperiod during plant ontogenesis and functioning of photoperiod-sensitive fertility restoring gene. Whereas, the ontogenetically-regulated expression of male fertility in both revertants was temperature-dependent and was clearly manifested under relatively cool conditions during 2-week period before

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the beginning of anthesis of the first panicle (average daily temperature 21⁰C). The increase of the average daily temperature by 2-3⁰C resulted in sharp increase of male fertility level. Possibility of using AS-1 line in a new "two-line system" of hybrid seed production, which require only two lines (sterile mutant and fertility restorer), is discussed.

Key words: CMS, fertility reversions, fertility restoring genes, male sterility, temperature-sensitive mutants, ontogenesis, sorghum, *Sorghum bicolor* (L.) Moench, ORMS – ontogenetically-regulated male sterility

INTRODUCTION

Among diverse sources of male sterility the mutants with variable expression of this trait altering in different environmental conditions attract a special attention. In past few years such mutants have been isolated in a number of crops, in rice and wheat, in particular. In these mutants the level of male fertility varies depending on the daylength duration (YUAN *et al.*, 1993; MURAI, TSUNEWAKI, 1993; OARD, & HU, 1995) and/or temperature regime (KATO *et al.*, 1990; SUN *et al.*, 1993) before and in the course of microsporogenesis. Alteration of these parameters during plant ontogenesis induces formation of partially or completely fertile tillers in male sterile plants. Such thermo- or photoperiod-sensitive mutants are of great interest both for investigating genetic control of plant male fertility and for development of so called "two-line systems" of hybrid seed production (JIN *et al.*, 1988; LOPEZ, VIRMANI, 2000). Contrary to cytoplasmic male sterility (CMS), these systems include only two lines - sterile mutant with environmentally regulated level of male fertility and fertility restorer - because there is no need to grow a special line-maintainers for propagating these mutants.

Previously, we reported on obtaining AS-1 line characterized by partial male fertility that varied in different panicles of one and the same plants and has unusual inheritance pattern (ELKONIN *et al.*, 1995). This line was developed after repeated self-pollination and selection of plants capable for seed setting in the progeny of regenerant obtained from tissue culture of a sorghum CMS plant. In this paper we report on alterations of expression of male fertility during ontogenesis of AS-1 plants and in the progeny of similar revertant ('124-1') obtained from fertile tillers, which developed on CMS-plant transferred from the field to the greenhouse. In addition, we demonstrate that fertility level in AS-1 line is probably influenced by an air temperature during the period of anther and pollen development and that this line is a temperature-sensitive nuclear male-fertile revertant.

MATERIALS AND METHODS

AS-1 line was obtained as a result of repeated self-pollination and selection for 7 generations of plants capable for seed setting in the progeny of a regenerant obtained from tissue culture of a sorghum CMS plant. This line is characterised by small and pointed anthers; on the bagged panicles the stigmas remain "fresh" for several days after the end of the blooming; nevertheless, seed setting takes place in the bottom of the panicle.

Revertant '124-1' was obtained from fertile tillers, which developed on a CMS plant found in F₂ population of a cross A1 Saratovskoye-3 / KVV-52, and transferred from the field to the greenhouse. Using previously developed methods (ELKONIN *et al.*, 1984), the embryogenic calli and plant-regenerants have been obtained from the shoot tiller of this plant. Regenerants as well as the donor plant were grown in the greenhouse. In the next season the donor plant has been transferred in the pot to an open air.

The progeny obtained from fertile tillers of this plant, as well as the plants of the AS-1 line were grown in the field in 3-5 m rows (5-6 plants / m). The plants of AS-1 line were grown also in the pots in the growth chambers under the short (12 h day / 12 h night) or long (16 h day / 8 h night) photoperiod (t⁰ 26-28⁰ C / 18-20⁰ C).

The level of male fertility was estimated on the panicles bagged before anthesis by staining pollen grains with 1% of I₂-KI and by seed setting level. Four samples were taken from two different branches from each panicle layer (middle and bottom). 200 pollen grains isolated from 5 anthers taken randomly from 5-7 flowers were scored in each sample. In AS-1 line pollen fertility of 8 plants bearing 2 panicles were estimated for each growing conditions. In '124-1' revertant pollen fertility was scored in 16 plants bearing 2 panicles, 12 plants with 3 panicles, 5 plants with 4 panicles and 3 plants with 5 panicles. Depending on the seed setting level the panicles were classified as sterile (s) (0% of seed setting), partially sterile (ps) (1-25%; approx. basal ¼ part of the panicle), partially fertile (pf) (25-75%; approx. ¾ of the panicle) and fertile (f) (> 75%; more than ¾ of the panicle). The plants were classified as sterile, partially sterile, partially fertile and fertile depending on the seed setting level on the main tiller.

To study inheritance of male fertility the intensively pollen-shading shoot tillers were crossed with progenitor CMS-line A1 Saratovskoye-3. This line is stable and never set seed on the bagged panicles.

The one- and two-factorial variance analysis, as well as χ^2 and Student's test were used for statistical evaluation of experimental results (ZAITSEV, 1984).

RESULTS AND DISCUSSION

Tissue culture-induced revertant (AS-1) - The plants of AS-1 line are characterized by variable expression of male fertility, the level of pollen fertility sometimes may vary in different flowers of one and the same panicle branch, in different panicle layer, in different tillers. Under growing in field conditions the main tillers usually are almost completely male sterile, whereas panicles on the shoot tillers possess significantly higher level of fertile pollen grains and could set seed. All panicles are characterized by clear basipetal gradient of male fertility: lower layers have much more higher per cent of fertile pollen grains in comparison with the middle one; in the upper layers the anthers are either absent or contain few sterile pollen grains (Table 1).

Table 1. Expression of pollen fertility in plants of AS-1 line growing in different environmental conditions

Growing conditions	Number of panicle	Pollen fertility (%) in layer of panicle ¹			F-criterion	
		Middle	Base	Average	Layer	Panicle
Field	1	6.0 a	13.7 b	9.9 a	9.66**	279.23***
	2	41.4 c	46.3 d	43.9 b		
	3	45.6	53.8	49.7		
	Average	23.7 a	30.3b	26.9		
12/12 ²	1	6.8 a	13.5 a	10.1 a	4.68*	13.03*
	2	23.5 a	46.8 b	35.2 b		
	Average	15.2 a	30.2 b	23.7		
16/8 ³	1	24.4 a	40.3 ab	32.4	8.59*	0.52
	2	26.2 a	47.6 b	36.9		
	Average	25.3 a	44.0 b	34.6		

¹ – per cent of fertile pollen grains; each value under each growing condition is a mean of estimation of 8 plants (data for panicle N3, which developed in field growing conditions, are mean of 3 plants). Under the same growing conditions data followed by different letters are significantly differed at 5% level according to Dun-can's Multiple Range Test. Data for panicle N3 were not included in the two-factorial variance analysis; ^{2,3} - plants were grown simultaneously in the growth chambers under 12 and 16 hours of daylength, correspondingly (t° 28-30/18-20 °C); *p<0.05; ** p<0.01; *** p<0.001

To study inheritance of partial male fertility of AS-1 line, it was crossed with progenitor CMS-line A1 Saratovskoye-3 and standard testers-fertility restorers. In the F₁ generation of crosses A1 Saratovskoye-3/AS-1, the partially sterile and sterile plants were observed; the sterile F₁ hybrids produced secondary partially sterile tillers (Table 2). This data testified that partial male fertility of AS-1 line is transmitted through the pollen and is controlled by dominant nuclear gene(s) participating in genetic control of CMS. However, in the F₂ generation unusual segregation ratio was observed, the sterile plants significantly predominated. This deviation could be explained by modified influence of environmental factors that hampers determination of a real number of nuclear genes participated in genetic control of partial male fertility. It should be noted that in the self-pollinated progeny of paternal plant grown in this year also predominated sterile and partially sterile plants (Fig. 1a). The appearance of only sterile F₁ hybrids in the cross of male sterile AS-1 plant with the line Saratovskoye-3B (sterility maintainer of progenitor CMS-line) testified to the genetic identity of the CMS-controlling genes of AS-1 and A1 Saratovskoye-3 cytoplasm.

Table 2. Fertility behavior of AS-1 line and its test-crosses with progenitor CMS-line A1 Saratovskoye-3, its fertile analogue Saratovskoye-3B and KVV-181, A1 CMS fertility restorer.

Progeny / Cross combination	Generation	Year	Number of plants ¹			
			f	pf	ps	s
AS-1	R ₅	1991	-	4	8	-
AS-1	R ₆	1992	1	-	5	8
A1 Saratovskoye-3 / ps from R ₆	F ₁	1993	-	-	4	5
Ps from R ₆ , self-pollination	R ₇	1993	7	9	8	-
A1 Saratovskoye-3 / ps from R ₆	F ₂	1994	-	1	7	54
Ps from R ₆ , self-pollination	R ₈	1994	-	1	10	16
AS-1 ³ / Saratovskoye-3B	F ₁	1994	-	-	-	10
AS-1 ³ / KVV-181 R	F ₁	1994	17	-	-	-

¹ s – sterile (0% of seed setting), ps – partially sterile (1-25%; approx. ¼ part of the panicle), pf – partially fertile (25-75%; approx. ¾ of the panicle), f – fertile (> 75%; more than ¾ of the panicle)

As far as one of the factors varying during plant vegetation is a daylength (from 16 h at the end of June, during formation of the main panicle, to 15 h at the beginning of August, during formation of the last panicles on the shoot tillers), we undertook a special experiment on growing AS-1 line in constant conditions during whole vegetation. The plants were grown simultaneously in the growth chambers under the short (12 h) and the long (16 h) photoperiod. It was revealed that in those plants, which were grown at the short daylength, were observed the same differences in pollen fertility between panicles that were seen in natural conditions, the second panicle was significantly more fertile than the first one (Table 1). The same basipetal gradient of pollen fertility was also observed on all panicles. These data testify that difference in fertility level of different tillers of AS-1 is not caused by change of photoperiod during plant ontogenesis and functioning photoperiod-sensitive nuclear gene(s), as in similar mutants of rice and wheat (YAUN *et al.*, 1993; MURAI, TSUNEWAKI, 1993; OARD, HU, 1995), but, perhaps, are conditioned by the action of “slowly working” or developmentally-regulated fertility-restoring gene.

Table 3. Dependence of male fertility of AS-1 line from the total air temperature during 2-week period before anthesis

Male fertility (seed setting level on the bagged first panicle ¹)	Total air /average daily temperature during 2-week period before anthesis, t ⁰ C
Sterile	295.8 a /21.1
Partially-sterile	310.8 b /22.2
Partially-fertile	332.5 c /23.8
Fertile	332.9 c /23.8

¹ The panicles were classified as sterile (0% of seed setting), partially sterile (1-25%; approx. basal ¼ part of the panicle), partially fertile (25-75%; approx. ¾ of the panicle) and fertile (> 75%; more than ¾ of the panicle). Data followed by different letters are significantly differed at 1% level according to Student's t-test.

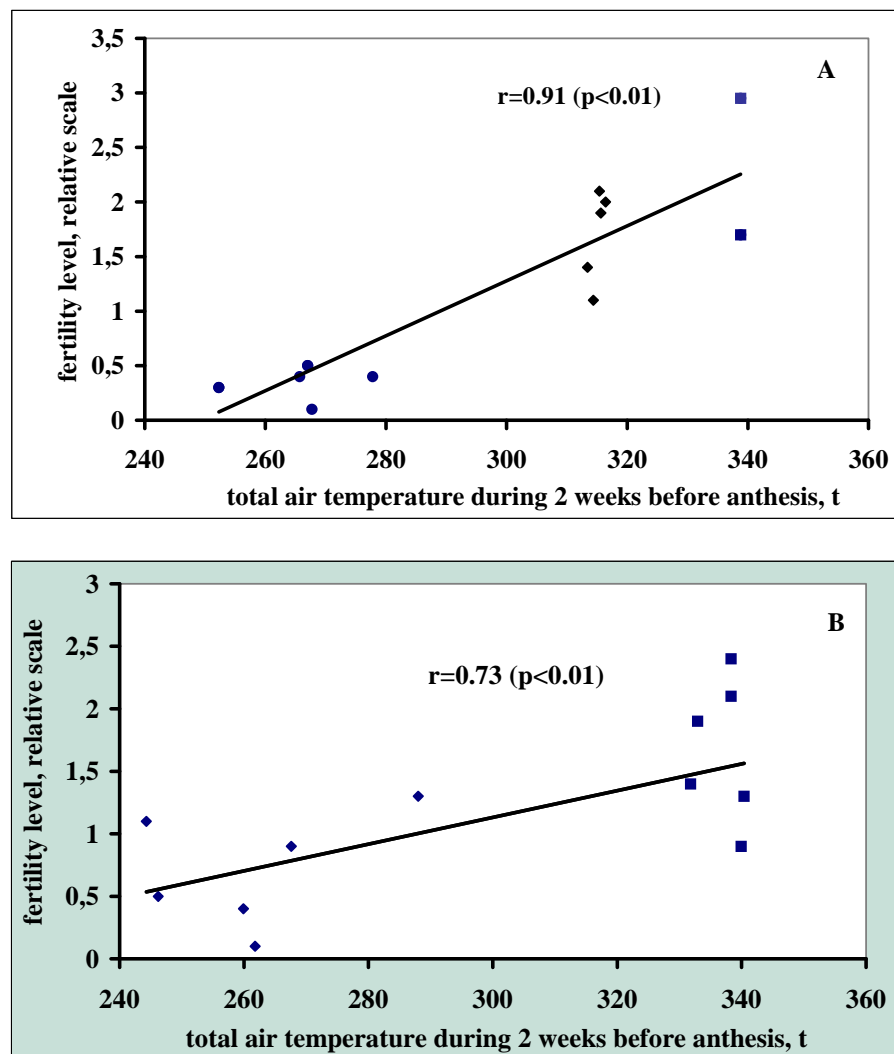


Figure 1. Dependence of the level of male fertility in the line AS-1 (A) and '124-1' (B) from the total air temperature during the 2-week period before anthesis. Each data is a mean seed setting level for an individual family; *s*, *ps*, *pf* and *f* plants were scored by the following scale: 0 – for steriles (absence of seed setting), 1 – for partially steriles (seed setting at basal $\frac{1}{4}$ part of the panicle), 2 – for partially fertiles (seed set at $\frac{3}{4}$ of the panicle) and 3 – for fertiles (seed set at more than $\frac{3}{4}$ of the panicle)

Another important environmental factor varying during plant vegetation is an air temperature. Careful analysis revealed a strong correlation between the AS-1 plants fertility level and the total air temperature during 2-week period before the

beginning of anthesis, i.e. during differentiation of microsporocytes, microsporo- and microgametogenesis ($r=0.91$; $p<0.01$). At relatively cool conditions when the average daily temperature during this period was about 21°C (296°C for 2 weeks) the plants formed male sterile panicles while the increase of the average daily temperature up to $23-24^{\circ}\text{C}$ ($310-330^{\circ}\text{C}$ for 2 weeks) resulted in development of predominantly fertile and partially fertile panicles. This correlation was observed both in one and the same season (Table 3) and in different seasons (Fig. 1a). Perhaps, such a temperature-dependent expression of male fertility explains the anomalous inheritance pattern of this trait in AS-1 line when self-pollinated fertile plants yield next season only sterile and partially sterile progeny (ELKONIN *et al.*, 1995).

Thus, the AS-1 line is a temperature-sensitive nuclear male-fertile revertant. Male fertility is conditioned by the action of "slowly working" fertility-restoring gene participating in the control of CMS. A product of this gene is gradually accumulated during plant ontogenesis and restores male fertility. In addition, this gene or its product is sensitive to a very fine shift of an air temperature during the period of anther and pollen development.

"Greenhouse-induced" revertant ('124-1') - In order to obtain reversions to male fertility of CMS-inducing genes in tissue culture, the embryogenic calli were induced from a shoot tiller of male-sterile plant ('124-1') chosen from the hybrid population $F_2 A_1$ Saratovskoye-3/KVV-52. For the maintenance of the donor plant, it was transferred from the field to the greenhouse. All 20 analyzed regenerants demonstrated complete male sterility when grown in the greenhouse, thus confirming the presence of genetic factors of CMS in the donor plant. However, after 6 months of growing in the greenhouse the fertile tillers appeared on the donor plant. Formation of fertile tillers continued next season in the open-air conditions.

In the progeny (S_1) grown from the seeds formed on fertile tillers, both partially and completely sterile plants were observed. In the next generation, fertile plants were already observed in the self-pollinated progeny of partially sterile plants. Inheritance of fertility reversion was tracked down to 6 cycles of self-pollination. This fact testifies that formation of fertile tillers in donor plant in the greenhouse was not caused by its growing conditions but was a result of genotypic changes.

Plants from different families and different generations were characterized by similar and highly specific expression of male fertility: fertile anthers were either absent on the main panicle or were located only in its lower layer, poorly dehisced and discharged pollen. Panicles of the shoot tillers possessed much more higher fertility level. Careful analyses revealed that, as in AS-1 line, panicle base possessed higher per cent of fertile pollen grains than the middle part, the upper part of the panicle usually had small wrinkled anthers with few sterile pollen grains. The later the panicle was formed in plant ontogenesis, the higher per cent of fertile pollen it had. Seed set data (Table 4) confirmed this regularity.

The level of pollen fertility of the main panicle also correlated with the rate of plant development. In the S_3 generation, the plants flowered on July, 22, had

significantly higher fertility level in comparison with more early flowered plants, on July, 11-20 (Table 5). Assuming extremely small change of a daylength during such a little time interval, one should suppose that this factor hardly could affect expression of pollen fertility in this revertant. At the same time, the plants flowered on July, 22, had significantly higher 2-week sum of air temperature before flowering than the early flowered plants. Comparison of air temperature sum for 2 weeks before anthesis showed that it significantly differed in plants with different fertility level (Table 5). A significant correlation was also observed under comparison of fertility level of '124-1' revertant and air temperature sum during 2-week period before anthesis in different seasons ($r=0.73$; $p<0.01$) (Fig. 1, B). Perhaps, in '124-1', as well as in AS-1, a «slowly working» temperature-sensitive gene is involved in synthesis of a product, gradually accumulating during plant ontogenesis and restoring male fertility.

Table 4. Fertility level of different panicles and different sectors of individual single panicle in revertant '124-1' (combined data from one S_3 , one S_4 and one S_5 families grown in the same year)

Panicle number	Fertility level					
	Pollen fertility, % ¹		Seed set, no. of panicles			Mean seed set level ²
	Middle	Base	s	ps	pf	
1 (main)	11.8 a	27.0 ab	9	7	0	0.4 a
2	24.6 ab	49.9 cd	3	3	8	1.4 b
3	39.4 bcd	57.2 d	0	3	9	1.8 b
4	44.4	62.4	0	2	4	1.7 b
5	50.6	62.7	0	0	3	2.0 b
F, panicle sector	15.474**					
F, panicle number	11.635**					

¹ Each value is a mean of 9 estimations (for panicles N1, N2 and N3), 5 (for panicle N4) and 3 (for panicle N5). Data followed by different letters are significantly differed at 5% level according to Duncan's Multiple Range Test. Data for panicles N4 and N5 were not included in the two-factorial variance analysis; ² Mean seed setting level was calculated as a sum of *s*, *ps*, *pf* and *f* panicles divided by total number of panicles; *s*, *ps*, *pf* and *f* panicles were scored by the following scale: 0 – for steriles (absence of seed setting), 1 – for partially steriles (seed setting at basal ¼ part of the panicle), 2 – for partially fertiles (seed set at ¾ of the panicle) and 3 – for fertiles (seed set at more than ¾ of the panicle). Data followed by different letters are significantly differed at 1% level according to Student's t-test. ** $p<0.01$.

To reveal genetic nature of fertility reversion, the pollen shading panicles of partially sterile plants were crossed with progenitor CMS-line A1 Saratovskoye-3. In the F_1 in all three crosses the fertile and partially fertile plants were observed (Table 6). These data show that fertility reversion is transferred through the pollen and, therefore, is conditioned by mutation of nuclear gene(s). Appearance of sterile plants in two of these test-crosses probably was due to heterozygosity of paternal plants and pointed on sporophytic mode of action of fertility-inducing gene(s).

Table 5. Dependence of fertility level of the main panicles of revertant '124-1' from the length of the period to the beginning of flowering and an air temperature (S_3 generation)

Male fertility (seed setting level on the bagged panicles)	Data of the beginning of flowering		Total air /average daily temperature during 2-week period before anthesis, t ⁰ C
	11.07–20.07	22.07	
Sterile	66	6	261.4 a /18.7
Partially-sterile	20	4	274.5 b /19.6
Partially and completely fertile	0	8	285.7 c /20.4
$\chi^2 = 42.28; p < 0.01$			

Data followed by different letters are significantly differed at 1% level according to Student's t-test. The total air temperature during 2-week period before anthesis for panicles with the same male fertility status was calculated by summing up the air temperatures for individual panicles and dividing the sum by the number of panicles with this fertility status. The average daily temperature during this period was calculated by dividing these values by the number of days (14).

Thus, expression of male fertility in revertant '124-1' has considerable similarity with the line AS-1. In both cases an ontogenetically regulated restoration of male fertility conditioned by nuclear genes is observed. These genes function in sterile cytoplasm and occurred as result of mutations in the course of propagation of CMS donor plants – either in tissue culture or *in vivo*. As far as these plants have been isolated from the F_2 populations, the appearance of fertility revertants may be due to genetic instability of heterozygous genotypes in conditions of artificial prolongation of their ontogenesis. It is well known that long clonal propagation induce changes in genotypes even in vegetatively propagated plants (SHARAFUTDINOVA, 2000). Perhaps, artificial conversion of annual sorghum plant into perennial one by its transfer to the greenhouse, induces changes, which in some extent are analogous to effect of tissue culture conditions. Among them are: activation of meristems suppressed during ontogenesis, change of endogenous phytohormonal balance, that could induce somatic mutations. Assuming the imbalance of hybrid genome, in hybrids this process may be more intensive than in homozygous genotypes.

Table 6. Fertility behavior of self-pollinated progenies of fertile tillers developed on plant '124-1' with CMS, which was taken from the field and transferred to the greenhouse, and their test-crosses with progenitor CMS-line A1 Saratovskoe-3

Progeny	Generation	Year	Number of plants			
			f	pf	ps	s
Self-pollinated revertant tillers	S_1	1993	-	5	9	13
A1 Saratovskoe-3 / <i>ps</i> -plant N1 from S_1	F_1	1994	7	16	18	-
A1 Saratovskoe-3 / <i>ps</i> -plant N2 from S_1	F_1	1994	2	5	-	19
A1 Saratovskoe-3 / <i>ps</i> -plant N3 from S_2	F_1	1994	12	3	7	4
<i>Ps</i> -plants from F_1^a	F_2	1995	9	2	5	12

It should be noted that genes controlling ontogenetically-regulated expression of male fertility in both revertants are temperature-sensitive and their effect disappears under alteration of temperature conditions during anther and pollen development. In this connection, these revertants have significant resemblance with the genic male-sterile temperature-sensitive mutants reported in rice (KATO *et al.*, 1990; SUN *et al.*, 1993). However, contrary to these mutants temperature-sensitive mutations in our revertants affected nuclear genes involved in control of CMS. In addition, in sorghum revertants the higher temperatures induced an increase of fertility level but not sterility as in rice mutants. The precise panicle sensitive developmental stage will be determined in future experiments.

As to our knowledge this is a first report on ontogenetically-regulated male-sterile mutants in sorghum. Taking into account a high level of pollen sterility of the first and often the second tillers of ORMS mutants it may be possible to develop a "two-line system" of hybrid seed production using them as a maternal lines. In our preliminary experiments conducted in different seasons under both artificial and open pollination of AS-1 panicles by a pollen of different sorghum lines in hybridization blocks, in the F₁ in many crosses were observed up to 80-100% hybrids. One of the hybrids (AS-1/KVV-181) showed high grain yield combined with early maturation and exceeded by these traits the standard F₁ hybrid Orion on the A1 type CMS. At the same time, the panicles of AS-1 line owing to their partial pollen fertility had a good seed set in the reproduction block. To avoid partial male fertility of AS-1 line and maternal seed set in hybridization block it may be promising an increase of seeding density that would suppress AS-1 tillering and, thus, prevent formation of partially fertile secondary panicles. After additional selection a non-temperature-sensitive genotypes could be obtained that would be stable in different environmental conditions.

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ONTOGENETSKI REGULISANA MUŠKA STERILNOST U KULTURI TKIVA – INDUKOVANI I SPONTANI MUTANTI SIRKA

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Izvod

U radu su prikazani rezultati paralelnih ispitivanja varijabilnosti ekspresije muške fertilitnosti u AS-1 liniji koja je dobijena somaklonalnim variranjem u kulturi tkiva biljke sterilne na bazi CMS (citoplazmatska muška sterilnost) i potomstva revertanta '124-1', dobijenog iz fertile metlice sa biljke premeštene iz poljskih uslova gajenja u kontrolisane uslove u staklari. Bez obzira na mehanizam restoracije sterilnosti ekspresija fertilitnosti je bila slična u toku ontogeneze: grančice na glavnoj stabljici metlice su bile kompletno sterilne a fertile polen je formiran na bočnim grančicama metlice. Jasan bazipetalni gradijent muške fertilitnosti je manifestovan na svim grančicama: osnova je imala značajno veći procenat fertile polenovih zrna u poređenju sa centralnim delom a na vršnom delu ili nije došlo do formiranja antera a ako je došlo do formiranja polenova zrna su bila sterilna. Restoracija fertilitnosti regulisana fazama ontogeneze je pod genetičkom kontrolom nuklearnog genoma i može da se vertikalno prenosi korišćenjem polena originalne linije prevedene na citoplazmatski sterilnu osnovu. Rezultati dobijeni gajenjem AS-1 biljaka u kontrolisanim uslovima u fitotronskim komorama uz simultano korišćenje uslova dugog (16/8) i kratkog dana (12/12) ukazuju da razlike u stepenu fertilitnosti nisu posledica različite dužine dana odnosno ekspresije fotoperiod – osetljivih restorer gena. Utvrđeno je da ontogenetski- regulisana reverzija fertilitnosti kod oba revertanta zavisi od temperature što je jasno manifestovano u uslovima relativno niskih temperatura dve nedelje pre početka pojave metlice (srednja dnevna temperatura oko 21 °C). Povećanjem srednje dnevne temperature za 2 – 3 °C došlo je do jasnog povećanja fertilitnosti polena. U radu je diskutovano o mogućnost korišćenja AS-1 linije u novom „dvolinijskom“ sistemu proizvodnje hibridnog semena, koji zahteva samo dve linije (sterilni mutant i fertile restorer).

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