

Evaluation of the combining ability of CMS lines in crosses with samples of grain sorghum and Sudan grass

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Abstract. The versatility of use, species diversity, and high drought resistance have ensured the demand for sorghum among agricultural crops in the world. Currently, the most promising direction of breeding is the creation of F1 sorghum hybrids, which is based on the identification and selection of parental forms with high combining ability. In this paper, the combining ability analysis crosses of two hybridization schemes: grain×grain sorghum and grain sorghum×Sudan grass was carried out using topcross method. Gene action governing inheritance of a particular trait can be measured in terms of general and specific combining ability estimates, where general combining ability effects are mostly indicative of additive gene action ($ms_{(GCA)}/ms_{(SCA)} > 1$) and specific combining ability effects - non-additive ($ms_{(GCA)}/ms_{(SCA)} < 1$). Genes with an additive effect participate in the genetic control of breeding-valuable traits, which is proven by $ms_{(GCA)}/ms_{(SCA)}$ ratios changing within 1.10-28.01 range. It is advisable to involve CMS-lines A3 Feterita 14, A2 KVV 114 and A2 O-1237 to create high-yielding hybrids of grain sorghum; highly productive sorghum-sudan grass hybrids - lines L-106, L-143 and Anastasiya, Kinelskaya 100, Elegiya variety samples; with a high share of grain in the total biomass – Allegoriya, Krasnodarskaya 75 and Zonalskaya 6 variety samples. CMS lines are distinguished by high and average CA values both in crosses with samples of grain sorghum and Sudan grass: by weight of 1,000 grains - A2 O-1237; by seed yield and weight of 1,000 grains - A2 KVV 114.

Key words: sorghum, Sudan grass, CMS lines, CMS types, F1 hybrids, GCA effects, SCA dispersion, agronomic traits.

INTRODUCTION

Sorghum is one of the most popular agricultural crops in the world, such as wheat, rice, corn, and barley. In Africa and Asia, sorghum is not only a forage crop, but also a food crop (Hariprasanna & Rakshit, 2016). In Russia, most of the acreage under sorghum is formed in the Volga, Southern and North Caucasus Federal Districts. The main use of grain sorghum is feeding, grass and sugar sorghum - forage. This crop is characterized by drought resistance, ability to form a sufficiently high productivity in the conditions of climate change in the direction of aridization (Pannacci & Bartolini, 2018; He et al., 2020). Therefore, the genetic improvement of the source material for use in increasing

the productivity of grain and biomass remains relevant, as well as the quality of the resulting products, especially in arid regions around the world.

Currently, breeding work is aimed at creating heterotic F1 hybrids. It should be noted that sorghum hybrids are characterized by superiority over the parental forms for productivity and other important agronomic traits. The heterosis effect has been widely used by breeders for a long time (He et al., 2020). For large-scale production of hybrids, it is necessary to use lines with cytoplasmic male sterility (CMS). Therefore, the determination of the general (GCA) and specific combining ability (SCA) of the components of the cross scheme is an important stage of breeding for heterosis (Justin et al., 2015; Kibalnik, 2017). GCA is understood as the average value of the parent line in combinations of crosses with its participation. The effect of GCA is determined by the action of additive genes. SCA evaluation allows for the characterization of each hybrid combination separately, when they turn out to be worse or better than expected based only on the effects of the GCA of the parent forms in the crossing scheme; it is controlled by the dominant and epistatic action of genes (Kumar et al., 2013; Khotyleva et al., 2016). It is noted that the components of crosses with high values of GCA effects are sources of agronomically valuable traits. Moreover, such parental forms are more adapted to the growing conditions (Fasahat et al., 2016). Analysis of SCA effects allows to identify promising hybrid combinations. In sorghum breeding, test and diallel crosses are the most commonly used methods for assessing the combining ability (Justin et al., 2015; Patil & Kute, 2015; Rocha et al., 2018; Da Silva et al., 2020). Thus, the analysis of male and female forms makes it possible to include the working collection more effectively in breeding programs on identification of heterotic hybrid combinations, to determine the inheritance of the analyzed traits and the action of genes/groups of genes that control the studied traits (Zhuzhukin et al., 2017; Oliveira et al., 2019).

Special attention should be paid to the use of different CMS sources in breeding in order to expand the genetic diversity of first-generation hybrids (Kibalnik & Semin, 2018). Cytoplasm A1 (*milo*) is the main and most common type of sterility in sorghum, used in breeding programs for the creation of grain and feed hybrids (Reddy et al., 2009; Aruna et al., 2012). Alternative types of CMS are included in the crossing schemes less frequently and are not fully studied (Kibalnik, 2017). Therefore, the purpose of the research was to evaluate the combining ability of CMS lines based on sterile cytoplasm A1, A2, A3, A4, 9E, M-351A in test crosses with samples of grain sorghum and Sudan grass.

MATERIALS AND METHODS

Field studies were conducted at the experimental field of the Institute in 2015–2018 (city of Saratov, Russia). The experimental area is characterized by the extreme continental climate. The temperature regime and water availability conditions are uneven. Frequent occurrence of soil and air drought is a peculiarity of the summer period. Average yearly precipitation varies between 250 and 450 mm, evaporation for the period from April to October amounts to from 450 to 770 mm. An average of 170–175 mm of precipitation falls within the vegetation period of sorghum. Yearly average air temperature amounts to +4.8 °C. The absolute temperature minimum is observed in January (-40 °C), maximum - July-August (+42 °C). The sum of active temperatures (over +10 °C) for sorghum active vegetation period amounts to 2,400–3,100 °C. In 2015, the hydrothermal coefficient was 0.41, which indicates a strongly pronounced lack of natural moisture

supply for plant development. In 2016–2018, the vegetation of hybrids and parental forms took place in ‘arid’ conditions ($HTC = 0.64\text{--}0.90$). The sum of active temperatures during the study period varied in the range of $2,613.1\text{--}2,805.0\text{ }^{\circ}\text{C}$, and the amount of precipitation - $106.2\text{--}178.3\text{ mm}$. Weather conditions for the study seasons were different (Fig. 1).

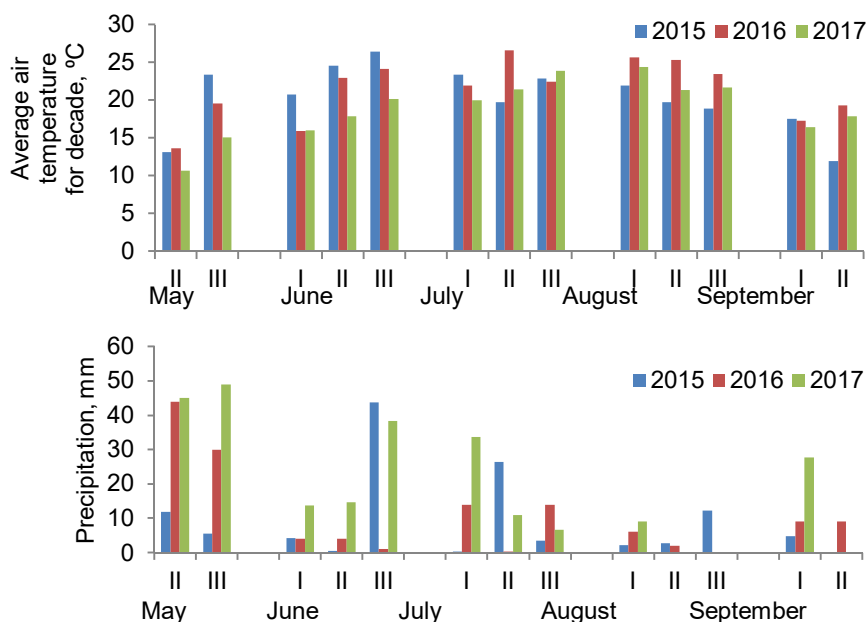


Figure 1. Meteorological conditions during the growing season of sorghum (2015–2017).

The soil of the experimental site is represented by southern medium-loamy chernozem. The humus content in the arable layer is 3.5%. Nitrification ability - 7.7 mg kg^{-1} ; phosphorus - $34.2\text{--}35.7\text{ mg kg}^{-1}$, potassium (in a carbon ammonium extract) - $349\text{--}378\text{ mg kg}^{-1}$. Reaction of the soil environment $\text{pH} = 6.3\text{--}6.4$. Content of mobile trace elements, mg kg^{-1} : sulfur - $6.0\text{--}6.1$, manganese - $4.6\text{--}5.5$, copper - $0.07\text{--}0.11$, zinc - $0.25\text{--}0.27$; boron - $1.60\text{--}1.75$.

The cultivation technology included the main technological operations: plowing, pre-sowing cultivation, sowing, post-sowing rolling, three row-to-row cultivations (the last one with hilling), harvesting. The predecessor is fallow. F_1 hybrids and parental forms were sown in a wide-row method with a row spacing of 70 cm in the third decade of May. The repetition in the experiment is threefold. The placement of plots with an area of 7.7 m^2 is randomized. The plant stand was set manually - 100 thousand pl. ha^{-1} . Traits' evaluation and the yield accounting were carried out according to the method of the state testing of agricultural crops (Methods of state variety testing of agricultural crops, 1989). The combining ability of the parental forms was determined by the topcross method (Savchenko, 1973). The following traits were analyzed: plant height, panicle length, panicle stem extension, weight of 1,000 grains, seed and biomass yield.

In this paper, two schemes of test crosses were considered. The first hybridization scheme (2015–2017) is aimed at creating a highly productive hybrid of grain sorghum. F_1 hybrids (49 in total) were obtained on the basis of seven CMS lines: A1 O-Yang 1, A2 KVV 114, A2 O-1237, A3 Feterita 14, A4 KP 70, M-35-1A Pishchevov 614,

9E Pishchevov 614 (Elkonin et al., 1997). Seven varieties were used as pollinators (testers): Mercury, Ogonyok, Avans, Topaz, Volzhskoe 615, Pishchevov 35, Volzhskoe 4.

In order to develop a productive sorghum-Sudangrass hybrid, a second hybridization scheme was created (2016–2017), including three CMS lines (testers) of grain sorghum - A2 O-1237, A2 KVV 114, A1 Efremovskoe 2 and 17 pollinators of Sudan grass - Zonalskaya 6, Chishminskayarannyaya, Krasnodarskaya 75, Kinelskaya 100, Zernogradskaya, Yaktash, Yubileynaya 20, Saratovskaya 1183, Zemlyachka, Allegoriya, Ambitsiya, Anastasiya, Faina, Elegiya, L-106, L-143, MEV-728.

Statistical processing of experimental research results was performed by a factor analysis of variance using the program 'Agros 2.09'.

RESULTS AND DISCUSSION

First generation hybrids' testing is carried out to obtain results on parental forms' combining ability (Khotyleva et al., 2016). The determination of the combining ability of the parental forms by test crosses method takes place in two stages: first, the differences between the hybrids in this crossing scheme are determined, and then the effects of GCA and SCA dispersion in the cross components (Savchenko, 1973; Zhuzhukin et al., 2017). The results of the dispersion analysis in the first scheme of crosses showed significant differences of F1 hybrids for all traits except for seed yield. However, it was established that it indicated a significant influence of the pollinator on the manifestation of all the studied traits ($p \leq 0.05$). There was no significant influence of the CMS-line and the interaction of factors (CMS-line \times pollinator) on the formation of grain yield (Table 1). When calculating the combining ability of parental forms, variability of F1 hybrids obtained on their basis was also reflected in a number of publications (Justin et al., 2015; Kibalkin, 2017; Oliveira et al., 2019).

Table 1. Variance analysis of the combining ability of sorghum CMS-lines for economically valuable traits (2015–2017)

Source	df	Plant height	Panicle length	Panicle stem extension	Weight of 1,000 grains	Seed yield
F _(hybrid)	48	8.03*	2.11*	2.50*	33.80*	0.90
F _(CMS-line)	6	10.98*	2.35*	3.09*	6.44*	1.49
F _(pollinator)	6	43.88*	10.59*	11.38*	18.24*	2.20*
F _(CMS-line \times pollinator)	36	1.56*	0.66	0.92	1.28	0.59
ms		87.06	9.48	17.51	8.34	2.26
F _(GCA CMS-line)	6	43.90*	10.59*	11.40*	18.35*	2.20*
F _(GCA pollinator)	6	10.98*	2.36*	3.09*	6.46*	1.48
F _(SCA)	36	1.56*	0.66	0.92	1.27	0.59
ms _(GCA)		1,274.32	33.50	66.54	51.08	1.66
ms _(SCA)		45.49	2.09	5.37	3.55	0.45
ms _(GCA) /ms _(SCA)		28.01	16.03	12.39	14.39	3.69

Note: * $p \leq 0.05$.

This table also contains significant effects of sterile lines' GCA by five main breeding traits ($p \leq 0.05$). The ms_(GCA)/ms_(SCA) ratios varied in the range of 3.69–28.01, which indicates the predominant influence of additive genes over non-additive ones in the genetic control of breeding-valuable traits.

To a greater extent, test crossings by top cross method allow to allocate parental forms with high GCA, whereas the system of diallelic crossings gives the fullest information on combining ability (Khotyleva et al., 2016). High GCA components effects of crossings indicate their value as a parental form and possess the most practical interest in the creation of perspective hybrids (Patil & Kute, 2015). In our research, CMS line on the basis of sterile A2 cytoplasm with positive GCA effects by a number of economic traits was allocated. The female form A2 O-1237 is characterized by an average common general combining ability in plant height (4.17), panicle stem extension (0.89), weight of 1,000 grain (0.36), seed yield (0.63), but low for panicle length (-0.49) (Fig. 2). It should be noted that literary data also contain information on Sudan grass CMS lines with high GCA on a complex of main selection traits - panicle length, panicle mass, weight of 1,000 grains, seed yield (Rafiq et al., 2002; Kibalnik, 2017).

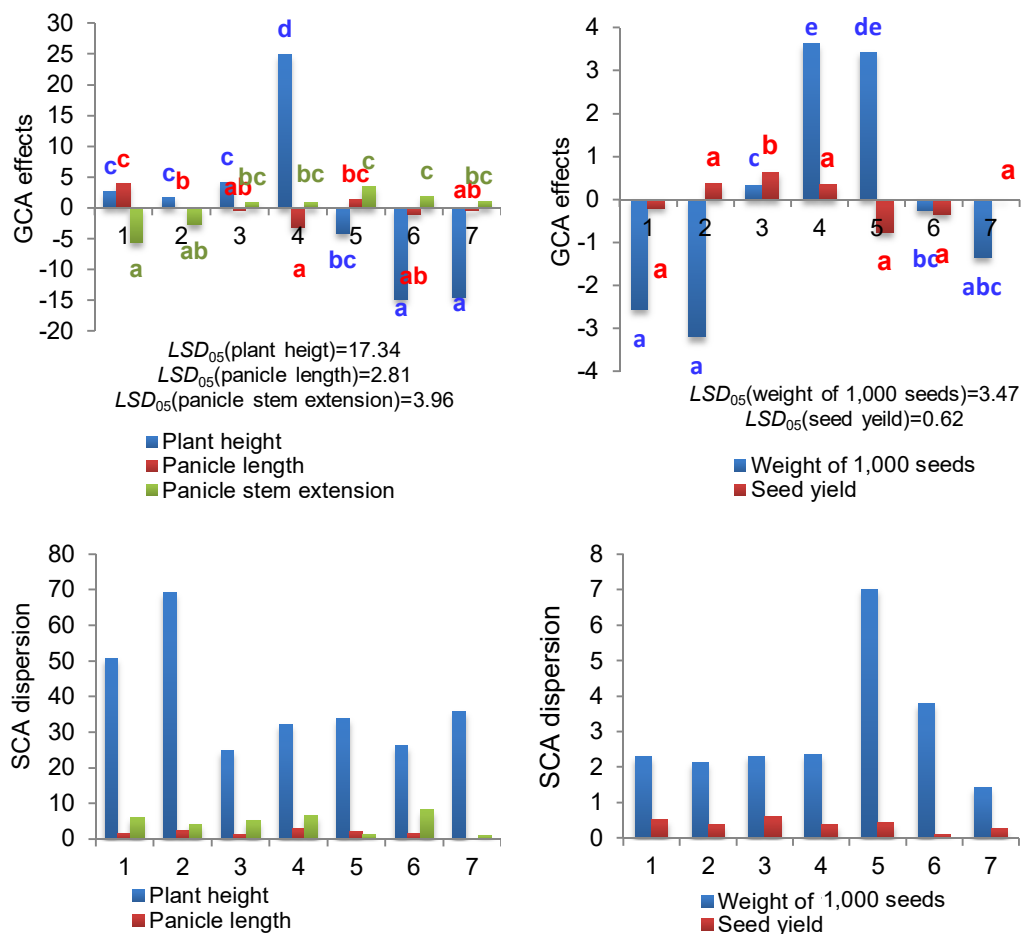


Figure 2. Effects of GCA and SCA dispersion of sorghum CMS-lines (2015–2017).

Note: 1. A1 O-Yang 1; 2. A2 KVV 114; 3. A2 O-1237; 4. A3 Feterita 14; 5. A4 KP 70; 6. M35-1A Pishchevoye 614; 7. 9E Pishchevoye 614. Data followed by the same letter did not differ significantly ($p \leq 0.05$) according to Duncan Multiple Range Test.

High SCA dispersion values for most of the studied traits were observed in CMS-lines A1 O-Yang 1 and A2 KVV 114. Grain sorghum hybrids when used on grain forage should be cultivated in a region with a plant height of no more than 130–150 cm. Therefore, the parental forms A4 KP 70, M-35-1A and 9E Pishchevoye 614 with low GCA effects (-14.78 – -4.11) are promising for such hybrid breeding programs. The breeding value of the CMS-line A4 KP 70 also lies in the fact that in crosses with individual male forms, the offspring has a fairly large grain: the SCA dispersion for weight of 1,000 grains was 7.02. Evaluation of the combining ability of CMS-lines showed that A3 Feterita 14 is characterized by high GCA effects in plant height (24.88), weight of 1,000 grains (3.62) and seed yield (0.34) and average SCA dispersions - 32.28; 2.34; 0.38, respectively (Fig. 2).

The results of the variance analysis confirm the significant differences between sorghum-Sudangrass hybrids ($p < 0.05$), as well as the significant influence of the male and female forms, their interaction for the manifestation of all the studied traits annually. It was found that in the genetic control of traits (weight of 1,000 grains, seed yields and biomass yield) genes with an additive effect are involved: the ratio of standard deviations varied in the range of 1.10–6.60 (Table 2).

Table 2. Variance analysis of the combining ability of CMS-lines of grain sorghum and variety samples of Sudan grass by economically valuable traits (2016–2017)

Source	df		Biomass yield		Weight of 1,000 grains		Seed yield	
	2016	2017	2016	2017	2016	2017	2016	2017
F _(hybrid)	41	35	154.03*	121.59*	129.22*	82.10*	29.11*	101.07*
F _(CMS-line)	2	2	345.10*	3.48*	864.24*	386.49*	40.40*	138.34*
F _(pollinator)	13	11	220.62*	174.03*	108.62*	104.09*	30.41*	227.73*
F _(CMS-line× pollinator)	26	22	106.03*	106.11*	82.98*	43.44*	27.59*	34.35*
ms	82	70	0.51	0.63	0.23	0.43	0.16	0.05
F _(GCA pollinator)	13	11	220.44*	173.96*	108.64*	104.01*	30.49*	228.56*
F _(GCA CMS-line)	2	2	344.81*	3.50*	864.47*	386.28*	40.48*	139.35*
F _(SCA)	26	22	105.95*	106.06*	82.99*	43.40*	27.66*	34.54*
ms _(GCA)			37.11	36.59	8.19	14.80	1.66	3.43
ms _(SCA)			17.84	22.31	6.25	6.18	1.51	0.52
ms _{(GCA)/ms_(SCA)}			2.08	1.64	1.31	2.39	1.10	6.60

Note: * $p \leq 0.05$.

Sorghum-Sudangrass hybrids are used not only for green forage, but also for grain storage, silage. For this purpose, the yield of F1 hybrids is accounted in the wax ripeness phase. Of the male forms, the high combining ability for the productivity of the entire biomass yield for 2016–2017 was established in the line of Sudan grass L-106 of the institute's breeding: the GCA effects were 5.66–7.84, the SCA dispersion - 4.25–57.68 (Table 3). The highest SCA dispersion in some years were also characterized by the variety samples Anastasiya, Kinelskaya 100, Elegiya (21.68–116.39) and the line L-143 (71.07). If the sorghum-Sudangrass hybrid is intended for grain-haylage, then it is necessary to take into account the share of seed productivity. In general, during the study period, a high combining ability for seed yield was established in the variety samples of Allegoriya, Krasnodarskaya 75 and Zonalskaya 6: the GCA effects varied in the range of 0.08–1.46; the SCA dispersion - 0.33–3.05. The variety samples of Zemlyachka, Chishminskayarannyaya and the line MEV-728 in 2016 were characterized by a high

specific combining ability (2.86–3.58). In 2016–2017, the variety sample Saratovskaya 1183 was distinguished by an average combining ability by weight of 1,000 grains: the GCA effects - 0.17–3.49, and the SCA dispersion - 0.55–4.03. In some years of the test, high CA for this trait was observed in Yaktash, Zemlyachka, and Elegiya: 1.44–2.62 and 7.31–25.29, respectively.

Table 3. Combining ability of components of sorghum-Sudangrass hybrids crosses according to the main agronomic traits, 2016–2017

Variety sample	Biomass yield				Weight of 1,000 grains				Seed yield			
	2016		2017		2016		2017		2016		2017	
	GCA ¹	SCA ²	GCA	SCA	GCA	SCA	GCA	SCA	GCA	SCA	GCA	SCA
Variety samples of Sudan grass												
Saratovskaya 1183	1.97	2.76	-2.38	0.05	0.17	4.03	3.49	0.55	-0.74	1.63	2.35	0.81
Zonalskaya 6	0.04	4.01	–	–	0.27	7.56	–	–	0.92	0.52	–	–
Yubileynaya 20	-4.76	5.10	-4.06	8.26	0.84	4.33	-0.08	6.93	-0.44	0.16	0.10	0.02
Ambitsiya	2.70	36.24	–	–	0.64	11.03	–	–	0.13	0.22	–	–
Allegoriya	2.34	0.64	-1.23	5.80	0.31	0.84	-0.88	6.59	0.76	0.33	1.46	2.42
Elegiya	–	–	-0.80	116.39	–	–	2.62	25.29	–	–	-1.53	0.33
L-143	-2.59	71.07	-2.85	1.05	1.21	2.31	-1.81	0.06	-0.67	1.56	0.42	0.01
Faina	–	–	2.92	16.48	–	–	0.62	3.62	–	–	-0.90	0.46
MEV-728	-0.53	8.02	2.71	1.77	-1.13	13.76	1.49	12.01	-0.54	3.14	-0.20	0.02
L-106	7.84	4.25	5.66	57.68	-2.19	12.06	-4.78	8.93	1.23	0.29	-0.09	0.30
Chishminskaya rannaya	-3.59	21.36	–	–	-0.76	1.57	–	–	0.46	3.58	–	–
Krasnodarskaya 75	0.84	19.66	-0.82	13.69	-1.16	3.95	0.79	1.10	1.00	3.05	0.08	0.35
Kinelskaya 100	-2.44	54.42	–	–	-3.76	1.11	–	–	-0.93	0.26	–	–
Zernogradskaya	-4.62	2.69	-0.24	0.09	2.23	1.01	-1.94	1.41	-0.65	0.29	-0.94	0.004
Yaktash	-0.63	1.37	–	–	1.87	7.31	–	–	-0.04	1.63	–	–
Zemlyachka	3.43	0.27	-4.58	2.45	1.44	10.39	-0.54	1.32	-0.51	2.86	-0.79	0.06
Anastasiya	–	–	5.67	21.68	–	–	1.02	0.14	–	–	0.04	0.91
CMS-lines of grain sorghum												
A2 O-1237	-2.23	5.42	0.02	20.63	2.49	5.10	1.99	3.90	-0.45	0.92	0.45	0.26
A2 KVV 114	0.47	12.57	-0.26	9.38	-1.17	3.80	0.28	4.95	0.16	0.96	-0.09	0.52
A1Efremovskoe 2	1.76	17.68	0.24	14.61	-1.32	3.57	-2.27	3.50	0.29	1.13	-0.37	0.26

Note: ¹ GCA – GCA effects; ² SCA – SCA dispersion.

In addition, it should be noted that sorghum-Sudangrass hybrids based on A1 Efremovskoe 2 differed in biomass yield productivity every year. The positive GCA effects of the CMS-line were 0.24–1.76, and the SCA dispersion was 14.61–17.68. Hybrids based on A2 O-1237 were distinguished by weight of 1,000 grains. This line has high GCA effects (1.99–2.49) and SCA dispersion (3.90–5.10). CMS-line A2 KVV 114 was characterized by an average combining ability for productivity, and the manifestation of the GCA effects depended on the growing conditions.

The selection of cross components in breeding for heterosis is of exceptional importance since the hybrid productivity depends on the genetic potential of the parental forms. Therefore, the study of the combinational ability of the source material is a fundamental step in hybrid breeding. It should be noted that hybrid sorghum breeding in most cases is based on the use of CMS lines with a high combinational ability for morphological traits and yield elements. Nevertheless, they were obtained on the A1

cytoplasm (Mahdy et al., 2011; Patil & Kute, 2015). To expand the genetic diversity of the source material, it is proposed to involve sterile lines with different CMS types as parental forms (Kibalnik, 2017). Currently, a great number of CMS-inducing cytoplasm are known in sorghum (Reddy et al., 2005). The discovery of new sterility sources requires a study of their breeding value. This direction is reflected in the few works of researchers, in which the assessment of the combining ability of CMS lines based on three types of cytoplasm - A1, A2 and A3 is considered (Mohammed, 2009; Reddy et al., 2009; Aruna et al., 2012; Zhou et al., 2021).

The presented experimental data demonstrate the expedient inclusion of the studied CMS lines obtained on the basis of the following sterility sources - A1, A2, A3, A4, 9E and M-35-1A - into the crossbreeding programs for the breeding of heterotic F1 sorghum hybrids. As a result of the analysis of combining ability, parental forms with high GCA effects and SCA variances were identified. Involvement in hybridization of grain sorghum and Sudanese grass samples as paternal forms made it possible to identify two CMS lines with the greatest significance in practical breeding - A2 O-1237 and A2 KVB 114. Thus female line A2 O-1237 has high indicators of GCA effects (0.36–2.49) and SCA dispersions (2.31–5.10) by weight of 1,000 grains; A2 KVB 114 has average values for seed yield (-0.09–0.63 and 0.52–0.96, respectively) and weight of 1,000 grains (-1.17–0.33 and 2.31–4.95, respectively). Understanding of the inheritance of agronomic traits is also necessary to create hybrids with given traits. In our study, it was revealed that genetic control of breeding-valuable traits in these hybridization schemes is carried out by genes with an additive effect, as evidenced by the values of the ratios of standard deviations 1.10–28.01 (Tables 1 & 2). There is also information in the literature that productivity and its elements (for example, weight of 1,000 grains) are under the control of additive genes (Akata et al., 2017; Chikuta et al., 2017; Kibalnik, 2017).

CONCLUSIONS

Hybrids of sorghum crops using cytoplasmic male sterility are distinguished by high yield of biomass and grain, which is in demand by the agricultural branches of different regions of sorghum production. The analysis of the combining ability of the parental forms of grain sorghum and Sudan grass of the two crossbreeding programs allowed to identify components with a high general and specific combining ability. It was found that in both directions of test crosses, the genetic control of the studied breeding-valuable traits is carried out by genes with an additive effect. It is notable that the sterile lines A2 O-1237 and A2 KVV 114 were characterized by a high combinational ability in terms of productivity elements both in crosses with grain sorghum and Sudanese grass samples, which indicates their significant breeding value.

It is reasonable to create highly productive hybrids of grain sorghum on the basis of CMS-lines - A3 Feterita 14, A2 O-1237, A2 KVV 114. To breed a sorghum-Sudan grass hybrid with high biomass productivity, it is recommended to use two lines L-106, L-143 and three variety samples Anastasiya, Kinelskaya 100, and Elegiya as the male form. When using a hybrid for grain-haylage, growing with a high proportion of grain in the total biomass, it is advisable to include the varieties Allegoriya, Krasnodarskaya 75 and Zonalskaya 6 in the hybridization program. To increase the seed size, it is necessary to involve the variety samples Saratovskaya 1183, Yaktash, Zemlyachka, and Elegiya in the crosses. It should be noted that the greatest practical

significance in crosses with samples of grain sorghum and Sudan grass was established in two CMS-lines: A2 O-1237 has indicators of GCA effects (0.36-2.49) and SCA dispersions (2.31–5.10) by weight of 1,000 grains; A2 KVB 114 has average values for seed yield (-0.09–0.63 and 0.52–0.96, respectively) and weight of 1,000 grains (-1.17–0.33 and 2.31–4.95, respectively).

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