Scientific Papers. Series A. Agronomy, Vol. LXVII, No. 1, 2024
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

HETEROSIS BASED ON MALE STERILITY IN COTTON

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Abstract

Heterosis is a method of increasing productivity and is used in many crops. In cotton, heterosis is mainly related to yield and fiber length. Two male-sterile cotton lines ms 273 and ms 274 were included in crosses with five modern varieties: Chirpan-539, Natalia, Rumi, Helius and Nelina. Heterosis manifestations were found for productivity/plant (7.4-63.8%) and fiber length in the F_1 hybrids. The cultivar Chirpan-539 and Rumi variety, with high GCA for fiber length and high variances of SCA, emerged as suitable for heterosis selection of this trait. The crosses 273 × Nelina and 274 × Nelina, with the highest productivity\plant (39.5-42.1 g) and the highest heterosis (61.9-63.8%), the second one with high SCA effects, are of interest for the heterosis selection of productivity. The crosses 274 × Chirpan-539 and 274 × Natalia, with the longest fibers (28.9-29.3 mm) and heterosis (2.1-3.5%), the first one manifested high SCA effects, are of interest for the heterosis selection of fiber length. The results obtained are encouraging for the development of heterotic selection and practical use of heterosis in cotton based on male sterility.

Key words: *cotton, inheritance, combining ability, productivity, fiber length.*

INTRODUCTION

Heterosis selection or heterosis method is of great importance to increase productivity. Heterosis is used in many crops and finds significant application in practice. In cotton, heterosis is mainly related to yield and fiber length and occurs in intra- and interspecific crosses. Significant heterosis for seed cotton yield was reported by Kencharaddi et al. (2015), Pushpam et al. (2015), Udaya et al. (2023). In our country, in cotton, heterosis manifestations were studied in intraspecific (*G. hirsutum* L.) and interspecific (*G. hirsutum* L. × *G. barbadense* L.) crosses and a number of promising combinations were identified.

In cotton, hybrid seed production is economically unprofitable because of castration and pollination of flowers is done manually, which is very laborious and expensive. For this essential reason, many researchers have studied male sterility (genetically and molecularly) to solve the problems with manual labor and reduce costs of hybrid seed production (Nie et al., 2018; Li et al., 2021; Zhang et al., 2021; Ma et al., 2021; You et al., 2022).

It is known that there are genetic male sterility, functional male sterility and induced male sterility. Genetic male sterility is of three types - genetic (nuclear), cytoplasmic and cytoplasmic-genetic, and all three types occur in cotton (Singh et al., 2012). GMS is widely used for hybrid seed production in India (Raja et al., 2018).

Different male sterility systems have been studied and used to exploit heterosis in the USA, India, China and other countries (Raja et al., 2018; Garcia et al., 2019; Han et al., 2022; Zhang et al., 2023). It was found that temperature, photoperiod and other environmental factors have influenced on male sterility (Khan et al., 2020; Zhang et al., 2020; Li et al., 2022).

In order to obtain high-yielding and high-quality hybrids based on male sterility great attention was paid to studying the degree of heterosis, combining ability and inheritance of economically valuable traits (Singh, 2006; Stoilova, 2008; Stoilova et al., 2008; Stoilova, 2009a; 2009b; Solanke et al., 2015).

In India, the USA, Israel, China high-yielding cotton hybrids and technology for hybrid seeds have been introduced into production. Both components of genetic variance, additive and non-additive, are relevant to the inheritance of traits in cotton. In breeding programs, in the inheritance of quantitative traits, additive genetic variance is more important. Non-additive gene effects are of greater importance for heterosis selection. Many authors are of the opinion that SCA is important for heterosis selection, heterosis utilization and creation of

heterosis varieties. Overdominant inheritance of seed cotton yield was reported by Igbal et al. (2005), Ahmad et al. (2005), Latif et al. (2014). Non-additive gene effects controlled seed cotton yield and its elements in the studies of Khokhar et al. (2018). Deosarkar et al. (2009), Sarwar et al. (2011) found non-additive gene effects for fiber length and fineness, Singh et al. (2010) – for lint percentage, 2.5% staple length and micronaire.

Many researchers have applied the line × tester cross method in upland cotton to determine GCA and SCA, their effects, and the level of heterosis (Jatoi & Memon, 2016; Sajjad et al., 2016; Karademir et al., 2016; Khokhar et al., 2017; Ali et al., 2018).

Heterosis is extremely important for cotton, especially under our climate, with limited temperature sums and rainfall supply.

The aim of this research was to study the inheritance, heterosis and combining ability in male sterility based cotton hybrids with a view to identify the hybrid combinations with high heterotic effect and best parents for heterosis selection.

MATERIALS AND METHODS

The study was carried out in the experimental field of the Field Crops Institute in Chirpan, in 2021-2022. Two new male-sterile cotton lines ms 273 and ms 274 were included in crosses with modern cotton varieties: Chirpan-539, Natalia, Rumi, Helius and Nelina. The two male-sterile lines were created by crossing the male sterile line 108 and Darmi variety (ms 108 × Darmi) and were selected in F_5 generation. Both lines are characterized by sterile and fertile phases. Stamens are deformed and difficult to open and pollen is highly sterile until 2-4 pm, which can solve the problem of manual castration. In these lines fixation and restoration of sterility are not necessary. Both lines are very early, low productive, have small flowers (petals) and bolls, long fibers and very low lint percentage. The cultivars Chirpan-539 and Helius and Rumi variety are very early and high productive, Natalia variety has very good fiber quality, Nelina variety has high lint percentage. The two male sterile lines, used as females, and the varieties, used as males (pollinators), were crossed by applying the line × tester (2 × 5) method to obtain 10 hybrid combinations. Hand pollination was applied without castration of stamens (emasculation).

The F_1hybrids and their parental forms were sown randomized in 3 replications and a 10 m^2 harvest plot. 10 plants per replicate were observed. Productivity per plant (g) and mean fiber length (mm) were recorded. To determine the type of inheritance in F_1, the genetic parameters for dominance (d) and additivity (a) and their ratio – d/a were used (Genchev et al., 1975). Heterosis relative to better parent was determined. Methodology of Savchenko (1984) was applied to establish the general (GCA) and specific (SCA) combining ability.

RESULTS AND DISCUSSION

Results of the male sterility based F_1 hybrids test are presented in Table 1. Heterosis for the productivity per plant ranged from 7.4% to 63.8% and was conditioned by overdominance. Six crosses, five significant and one non-significant, exhibited positive heterosis over the better parent. The crosses ms 273 × Nelina and ms 274 × Nelina showed maximum heterosis values (61.9-63.8%) and the productivity per plant was the highest (39.5-42.1 g). The crosses ms 273 × Helius, ms 273 × Rumi and ms 273 × Natalya showed much less pronounced heterosis (11.7-19.3%) significant at $P \leq 0.05$ (for the first two) and $P \leq 0.01$. The productivity per plant in these ones was lower - 30.3-33.9 g.

In male sterility based hybrids, different levels of heterosis have been reported, depending on the type of hybrids (intraspecific or interspecific, tetraploid or diploid), the genotype of parental forms and their combining ability. In the research of Shashibhushan and Patel (2019) in CMS based upland cotton hybrids for seed cotton yield heterosis over the standard check ranged from -39.17 to 9.36 per cent. In GMS based diploid cotton hybrids the seed cotton yield exhibited heterosis which ranged from -5.47 to 51.28 per cent over the better parent and -5.07 to 28.26 per cent over the check (Solanke et al., 2015). In a study by Stoilova (2009a) in intraspecific *G. hirsutum* hybrids based on CMS the heterotic effect for productivity per plant over the better parent varied from 4.5 to 37.6 per cent, average for three years. Tuteja et al. (2005) applying GMS

system identified hybrids showed by 11.88-16.07% higher heterosis than commercial hybrid "LHH 114". Nirania et al. (2004a) announced 36.55% heterotic effect for seed cotton yield in GMS based hybrid IAN-579 × G-67. High heterotic effect of hybrid SA278 × A72-15 (32.36%) was confirmed in other research (Nirania et al., 2004b). In previous studies high heterosis for seed cotton yield was also reported by many authors.

In cotton, heterosis of 50% over the popular variety and 20% over the popular hybrid is considered significant for development of hybrid cultivars (Singh et al., 2012).

The crosses ms 273 × Natalia, ms 274 × Chirpan-539 and ms 274 × Natalia had the longest fibers (28.6-29.3 mm), with incomplete dominant (in the first cross) and overdominant inheritance to the better parent. Overdominance caused weak heterotic effect of 2.1-3.5%. At the other eight crosses, the inheritance of this trait was incompletely dominant to the parent with the lower or higher value and additive only in one cross. Shashibhushan and Patel (2019) reported heterosis over the standard check variety from -13.44 to -4.69 per cent in 2.5 per cent span length, in CMS based upland cotton hybrids. None hybrid showed positively significant heterosis over standard check variety.

Table 1. Inheritance and heterosis for seed cotton yield per plant and fiber length in F_1 male sterility based hybrids

Crosses	P_1	P_2	F_1	d/a	Heterosis
Productivity per plant. *g*					
ms 273 × Chirpan-539	24.4	30.8	23.3	-1.34	75.6
ms 273 × Natalia	24.4	25.4	30.3	10.80	119.3
ms 273 × Rumi	24.4	30.0	33.9	2.39	113.0
ms 273 × Helius	24.4	25.7	28.7	5.62	111.7
ms 273 × Nelina	24.4	23.5	39.5	34.56	161.9
ms 274 × Chirpan-539	25.7	30.8	29.7	0.57	96.4
ms 274 × Natalia	25.7	25.4	24.1	-9.67	93.8
ms 274 × Rumi	25.7	30.0	27.3	-0.26	91.0
ms 274 × Helius	25.7	25.7	27.6	-	107.4
ms 274 × Nelina	25.7	23.5	42.1	15.91	163.8
GD 5.0%; 1.0%; 0.1%	3.0; 4.2; 5.2				
Fiber length. *mm*					
ms 273 × Chirpan-539	29.0	26.2	27.0	-0.43	93.1
ms 273 × Natalia	29.0	26.9	28.6	0.62	98.6
ms 273 × Rumi	29.0	26.5	27.7	-0.04	95.5
ms 273 × Helius	29.0	25.4	26.0	-0.67	89.7
ms 273 × Nelina	29.0	23.7	26.6	0.09	91.7
ms 274 × Chirpan-539	28.3	26.2	28.9	1.57	102.1
ms 274 × Natalia	28.3	26.9	29.3	2.43	103.5
ms 274 × Rumi	28.3	26.5	27.7	0.33	97.9
ms 74 × Helius	28.3	24.4	27.5	0.59	97.2
ms 274 × Nelina	28.3	23.7	27.0	0.43	95.4
GD 5.0%; 1.0%; 0.1%	1.5; 2.0; 2.5				

The magnitude of heterosis for fiber length in this study was in accordance with that reported by Stoilova (2009a) the heterotic effect by this fiber property was slightly expressed from 2.7% to 7.0%, average for three years. Inheritance of fiber length was basically dominant to the better parent and in some crosses with weak positive overdominance. In another previous study (Stoilova, 2008) heterosis manifestations for fiber length were also weakly expressed, from 0.5% to 4.2%.

Combining ability of parental forms is of great importance for selection strategy in cotton. The GCA effects of male sterile lines used as mothers were insignificant for productivity per plant and weakly significant for fiber length, at $P \leq 0.05$ (Table 2). The GCA effects of males were significant, at $P \leq 1\%$, for productivity per plant and for fiber length, which means that they differed in general combining ability for both traits. The SCA effects were only significant for productivity per plant and not

significant for fiber length, meaning that the parental forms differed in specific combining ability only in productivity per plant and did not differ in fiber length.

Table 2. Analysis of variance of combining ability

Source of variation	Degrees of freedom	Sum of squares	Mean square	F experimental
Productivity per plant, g				
GCA - females	1	2.4678	2.4678ns	2.5682
GCA males	4	276.6172	69.1543	71.9682**
SCA	4	63.2607	15.8152	16.4587**
Errors	10		0.9609	
Fiber length - mm				
GCA - females	1	2.0557	2.0556	7.7856*
GCA males	4	6.9302	1.7325	6.5618**
SCA	4	1.1606	0.2902	1.0990ns
Errors	10		0.2640	

Of the two maternal forms, ms 273 showed positive but insignificant GCA for the productivity per plant (Table 3). Among the pollinators, only Nelina variety exhibited significant, high and positive GCA for the productivity per plant, while all the others had negative GCA, insignificant for Rumi variety. Stoilova et al. (2008), Stoilova (2009a), for productivity per plant in F_1 CMS based hybrids (*G. hirsutum* L.), reported positive GCA effects for Natalia and Chirpan-539, which was not confirmed in the present study.

Table 3. Evaluation of the GCA effects for productivity per plant and fiber length

Productivity per plant							
Females	CGA	Males	GCA	Females	GCA	Males	GCA
ms 273	0.4967	Chirpan-539	-4.1733	ms 273	-0.4533	Chirpan-539	0.3133
ms 274	-0.4967	Natalia	-3.4233	ms 274	0.4533	Natalia	1.3467
		Rumi	-0.0400			Rumi	0.0967
		Helius	-2.5067			Helius	-0.9033
		Nelina	10.1433			Nelina	-0.8533
M_{Di}	0.6200	M_{Dj}	0.9803	M_{Di}	0.3250	M_{Dj}	0.5138

As for fiber length, of the two maternal forms, ms 274 exhibited positive and significant GCA. From the paternal forms, Natalia variety, with introduced germplasm from the *G. barbadense* L. species, had the highest and significant GCA. The cultivar Chirpan-539 and Rumi variety, with shorter fiber than Natalia variety, had positive but insignificant GCA. The cultivar Helius and Nelina variety had significant negative GCA for this trait. In the studies of Stoilova (2008; 2009a) Natalia variety had positive GCA, which is confirmed in our research, while the cultivar Chirpan-539 had negative GCA effects, which cultivar in our study had insignificant positive GCA effects. SCA effects are of greater importance for heterosis selection. Five crosses (50%) had positive SCA effects for the productivity per plant (Table 4). The cross ms 274 × Chirpan-539 showed the highest positive SCA effects. Both parental forms had negative GCA effects, revealing non additive type of gene action. According to Khan et al. (2005), Khan et al. (2007), Soomro (2010) GCA was not a criterion for predicting SCA, GCA and SCA were different independent characteristics. The crosses ms 274 × Nelina, ms 273 × Natalia and ms 273 × Rumi, with high productivity per plant (30.3-42.1 g) and manifested heterotic effect, had high and positive SCA effects. In these crosses, one of the two parental forms had positive GCA effects, which is consistent with that reported by Zhang et al. (2016), Sivia et al. (2017), Vasconcelos et al. (2018) that crosses with significant SCA included at least one parent with high GCA. Among them the cross ms 274 × Nelina had the highest productivity (42.1 g) and the highest heterotic effect (63.8%).

Table 4. Estimation of the SCA effects (S_{ij}) and the variances ($\sigma^2 s_i$; $\sigma^2 s_j$) for productivity per plant and fiber length

Male	Chirpan-539	Natalia	Rumi	Helius	Nelina	$\sigma^2 s_i$
Females	Productivity per plant, g					
ms 273	-3.7133	2.6033	2.8200	0.0533	-1.7633	7.5233
ms 274	3.7133	-2.6033	-2.8200	-0.0533	1.7633	7.5233
$\sigma^2 s_j$	27.1933	13.1704	15.5205	-0.3787	5.8343	
	M_D=0.7593					
	Fiber length, mm					
ms 273	-0.4800	0.0877	0.4377	-0.2967	0.2533	0.0396
ms 274	0.4800	-0.0867	-0.4367	0.2967	-0.2533	0.0396
$\sigma^2 s_j$	0.3552	-0.0906	0.2757	0.0704	0.02274	
	M_D=0.3980					

Nelina variety, from the males, exhibited high GCA for this trait, had low variance of SCA, which shows that the high GCA was mainly due to additive gene effects and it is not very suitable for heterosis selection. The cultivar Chirpan-539 showed negative GCA had high variances of SCA. In previous studies by Stoilova et al. (2008), Stoilova (2009a) this cultivar was found to have positive GCA for productivity/plant and high variances of SCA and to be most suitable for the heterosis breeding.

Regarding fiber length, also five crosses, significant in three, exhibited positive SCA. The cross ms 274 × Natalia, with the longest fiber (29.3 mm) and the highest heterotic effect (3.5 %) showed insignificant negative SCA effects, while the other two crosses ms 273 × Natalia and ms 274 × Chirpan-539, with the same length of fibers (28.6 mm and 28.9 mm) exhibited positive respectively insignificant and significant SCA effects. Heterosis, however, was observed only in the second cross. In the first cross only Natalia variety was with high and positive GCA, while in the second one both parents exhibited positive GCA effects.

Natalia variety, with the highest GCA for fiber length, had very low variance of SCA and is not suitable for heterosis selection of this trait. The same conclusion was drawn by Stoilova (2008; 2009a) that Natalia variety with positive and high GCA for fiber length had very low variance of SCA and was nat sutable for use in the creation of high quality heterosis varieties. The cultivar Chirpan and Rumi variety, with positive, but insignificant GCA, had high variances of SCA, indicating that their GCA effects were due to both additive and non-additive (different gene interactions, dominance and epistasis) gene effects and are suitable for heterosis selection of fiber length.

Summarized results show that ms line 273 had positive GCA for the productivity per plant, while ms line 274 had positive GCA for the fiber length. From the pollinators, Nelina variety had high and positive GCA for the productivity per plant, Natalia variety was with high and positive GCA for the fiber length. This two pollinators, however, had low SCA variances and are not suitable for heterosis selection for these traits. The crosses ms 273 × Nelina and ms 274 × Nelina were found to be highly heterotic for the productivity per plant. The crosses ms 274 × Natalia, ms 274 × Chirpan-539 and ms 273 × Natalia were found to be promising for the fiber length, first two with heterotic manifestations for this trait. The expression of high heterosis for the productivity per plant and moderate one for the fiber length in some crosses reveal possibility for exploitation the heterosis and male sterility for developing of male sterility based cotton hybrids.

In connection with climate changes and global warming, the efforts of many researchers are directed to the development and implementation of economic and ecological technologies for cotton by reducing the irrigation, mineral fertilization, etc. (Gospodinova & Stoyanova, 2020; Muhova & Dobreva, 2022). An integral part of these technologies is the introduction of new cotton

varieties resistant to drought and responsive to intensive factors such as fertilization and irrigation. Vozhehova et al. (2022) assessing genetic diversity and population structure of cotton gene pool samples identified 30 sources of economic valuable traits with high adaptability and created two new cotton varieties. Gospodinova & Stoyanova (2020) found positive correlations of productivity and its elements, under different levels of mineral fertilization and moisture supply. The use of heterosis, based on male sterility, can play much more significant role in increasing cotton yield and overcoming existing negative correlations with fiber qualities. Muhova & Dobreva (2022) in their study found negative correlations of fiber length with all other traits. The results from this study and previous research (Stoilova, 2008; Stoilova et al., 2008; 2009a; 2009b) are a good prerequisite for the development of heterotic direction in the cotton breednig in our country. However, further research is needed on the genetic nature of sterility of the available sterile lines; to test a larger set of crosses in a larger number of environments (years); to transfer sterility to promising modern cotton varieties.

CONCLUSIONS

Some of the male sterility based F_1 hybrids exhibited high heterotic effect for the productivity per plant. The inheritance of fiber length was complete and incomplete dominant to the better parent and in individual cases it was positive overdominance with slightly pronounced heterosis.

Nelina variety with high GCA for the productivity per plant had low variance of SCA, which outlines it as more suitable for the synthetic (pedigree) selection.

The cultivar Chirpan-539 and Rumi variety, exhibited high GCA for fiber length and high variances of SCA, emerge as suitable for heterosis selection, while Natalia variety, with the highest GCA and low variance of SCA, is more suitable for synthetic selection.

The crosses ms 273 × Nelina and ms 274 × Nelina, with the highest productivity per plant (39.5-42.1 g) and the highest heterosis (61.9-63.8 %), the second one with high and positive SCA effects, are of interest for the heterosis selection of productivity.

The crosses ms 274 × Chirpan-539 and ms 274 × Natalia, with the longest fiber (28.9-29.3 mm) and heterosis (2.1-3.5%), the first one with high SCA effects, are of interest for the heterosis selection of fiber length.

The results obtained are encouraging for the development of heterosis selection in cotton.

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