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Genetics and Breeding

Breeding assessment of new promising cotton lines

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Abstract. *Cotton breeding in Bulgaria is mainly aimed at improving the earliness, productivity and fiber quality of modern varieties. The creation of new genetic diversity is one of the basic prerequisites for the success of breeding programs. The aim of the study was to evaluate cotton lines obtained by intraspecific and remote hybridization combined with backcross technology, with a view to their most effective usage in selection. Twenty-three lines were included in competitive variety trials conducted during the 2014-2017 period. The averaged results showed that lines 550, 639, 641, obtained by remote hybridization, appeared to be very promising. These three lines were distinguished by the best combination of productivity, fiber length and fiber lint percentage, and by these three indicators they exceeded the standard variety Chirpan-539. A new cotton variety Aida (No. 457) was approved, which in seed cotton yield and fiber yield, and technological fiber properties surpassed the standard varieties Chirpan-539 for earliness and productivity and Avangard-264 for fiber quality. The candidate variety 535 continued the state variety testing. Two new candidate cotton varieties No. 550 and No. 553 were released. In the state variety testing the three candidate varieties confirmed their qualities. The obtained lines, distinguished by one trait or by a complex of qualities, enriched the gene pool of Bulgarian cotton.*

Keywords: breeding, cotton, candidate varieties, fiber properties, yield

Introduction

The main task of each breeding is constantly to improve the existing varieties. Cotton breeding programs in Bulgaria are mainly aimed at improving the earliness, productivity and fiber quality of the modern varieties.

In the period 1994-2015, 24 new cotton varieties were created and approved at the Field Crops Institute in the town of Chirpan. Various breeding methods were used: to increase the earliness and productivity, intraspecific hybridization and experimental mutagenesis were essential, and to improve the quality of cotton fiber, interspecific hybridization of *G. hirsutum* L. × *G. barbadense* L. and its combination with intraspecific hybridization was used.

Chirpan-603 and Chirpan-539 varieties were a new stage in cotton breeding in Bulgaria for earliness and productivity. These varieties were earlier and more productive than the standard Beli Izvor variety and replaced it in production (Bozhinov et al., 1996). Beli Iskar, Beli Lom, IPTP Veno, Boyana and Denitsa varieties were a step forward in the cotton selection for increasing the productivity and fiber lint percentage (Bozhinov and Bozhinov, 2004, 2008; Valkova and Bozhinov, 2010; Valkova, 2014a). Intraspecific hybridization was used for the creation of these varieties.

Trakia, Helius, Philippopolis and Sirius varieties, created by the method of experimental mutagenesis, combine earliness, high productivity and a number of other valuable economic qualities (Valkova, 2009, 2014b, 2017).

Avangard-264, Perla-267, Vega, Colorit, Darmi and Natalia varieties, with *G. barbadense* L. genplasm, are of improved fiber quality in terms of its length, fineness and strength (Koynov and Stoilova, 1996; Stoilova and Saldzhiev, 2000; 2005a,b; 2008a,b; 2010). The success of any breeding program to a large extent depends on the available genetic diversity and its evaluation.

The aim of this study was to perform breeding assessment by the most important economic traits of cotton lines included in the competitive variety test with a view to their effective usage in the breeding programs.

Material and methods

In the competitive variety trial carried out in 2014-2017 eight lines obtained by applying different breeding methods were included: lines No. 426, No. 449, No. 457, No. 550 and No. 553 - by interspecific hybridization of the *G. hirsutum* L. with the wild diploid species *G. thurberi* Tod., *G. davidsinii* Kell. and *G. raimondii* Ulbr., and saturating backcrosses with the *G. hirsutum* L.; the rest of the lines (No. 346, No. 489 and No. 535) - through intraspecific diallel and line × tester crosses, as

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one of the parents included in its genotype *G. barbadense* L. germplasma.

In another experiment conducted in 2016-2018 two lines - No. 639 and No. 641 were included. Both lines were obtained by interspecific hybridization of the *G. hirsutum* L. species with the synthetic allotetraploid of *G. thurberi* × *G. raimondii* wild species and backcross technology.

In the third trial carried out in 2017-2018 new 13 lines obtained through intraspecific diallel (No. 668, No. 669, No. 671, No. 672, No. 673, No. 675 and No. 678) and double crosses (No. 652, No. 654, No. 657, No. 661, No. 662 and No. 664) were included. Only one line was obtained by interspecific hybridization.

The experiments were carried out in the experimental field of the Institute of Field Crops Institute - Chirpan, on leached vertisols type of soil, and were plotted by the block method, in 4 replications and a 20 m^2 harvest plot. Ten plants of replication were observed. The following were reported: yield of raw cotton from a plot; boll weight; fibre lint percentage and fibre length measured by the "butterfly" method. All tested lines were compared with the standard variety Chirpan-539.

The years of the study were characterized as follows: in terms of temperature security coefficient, 2015, 2016, 2017 and 2018 were warm (P=14.3-17.2%), 2014 was average to medium cool (P=66.7%); in terms of rainfall, 2015 and 2017 were moderately wet (P=28.6-33.3%), 2014 and 2018 were wet (P=12.9-14.8%) and 2016 was dry (P=93.1%). The diversity of the years in terms of the rainfall supply was greater compared to the temperature security.

P- temperature security coefficient determined on the basis of the order of the years in descending order, respectively by the temperature sum for May-September and the rainfall sum for May-August (P,%=n/m+1×100, where n was the order number of the year of testing; m – the total number of years included in the descending order of years - climatic norm). The period 1989-2018 (last 30 years) was considered the climatic norm. The climatic norm was the average value for a fixed base period of 30 years (Alexandrov et al., 2010). The base periods adopted so far by Alexandrov et al. (2010) were from: 1901-1930; 1931-1960; 1961-1990.

A two-factor analysis of variance was performed on the obtained results (Lidanski, 1998). The ANOVA 123 program software was used.

Results and discussion

The averaged results for 2014-2017 showed that six lines had significantly higher total seed cotton yield than the standard variety Chirpan-539, exceeding it by 7.2-11.1% (Table 1). The highest yield was found for line 535. Of the 8 lines tested, 7 showed longer fibers, exceeding the standard by 0.3-1.2 mm in fiber length. The longest fiber was found for line 449 - 27.1 mm at 25.9 mm for the standard. Three lines had significantly higher fiber lint percentage - 41.8-42.3%, compared to 41.0% for the standard.

Table 1. Agronomic qualities of lines in competitive variety testing for 2014-2017 (average for 4 years)

Line No.	Origin	Seed cotton yield kg/ha	In % to Chirpan-539*	Boll weight, G	Fiber length, mm	Fiber lint percentage, %
Chirpan-539*	Intraspecific hybridization	1615	100.0	5.2	25.9	41.0
346	Intraspecific hybridization	1656	102.5	4.9^{000}	26.2^{+++}	41.8^{++}
426	Interspecific hybridization	1731	107.2^{++}	5.1	25.7	40.9
449	Interspecific hybridization	1735	107.4^{++}	5.2	27.1^{+++}	39.9^{000}
457	Interspecific hybridization	1771	109.7^{+++}	4.9^{00}	26.4^{++}	39.5^{000}
489	Intraspecific hybridization	1531	94.8^{0}	5.2	26.9^{+++}	42.3^{+++}
535	Intraspecific hybridization	1794	111.1^{++}	5.2	26.6^{+++}	40.6
550	Interspecific hybridization	177.1	109.7^{++}	5.0^{0}	26.3^{+}	42.2^{+++}
553	Interspecific hybridization	1761	109.0^{++}	5.1	26.8^{+++}	41.0
GD 5.0%		81	5.0	0.2	0.3	0.5
GD 1.0%		107	6.6	0.3	0.5	0.7
GD 0.1%		138	8.5	0.4	0.6	0.9
Accuracy		3.39		2.99	0.93	0.93

*Standard

In a complex assessment, the following were outlined as the best lines: Number 550 - this line in raw cotton yield was superior to the standard by 9.7%. The higher productivity was combined with longer fiber of 0.4 mm and higher lint percentage of 1.1%. Number 535 - this line exceeded in seed cotton yield the standard variety by 11.1% and was superior to it in fiber length by 0.7 mm. In fiber lint percentage the line was equal with the standard. Number 535 was obtained by intraspecific hybridization from the crossing of breed line No.37 with Dorina variety (No.37×Dorina). Number 550 was created by interspecific hybridization and backcross technology, from the crossing of line 413 - *Gossypium hirsutum* L. (2n=52) with the wild diploid species *Gossypium davidsonii* Kel. (2n=26) and three times backcrossing of the amphidiploid (allohexaploid) (line 413 - *G. hirsutum* L. × *Gossypium davidsonii* Kel.) (2n=78), as the first backcrossing was applied in C_1, the next two backcrossings were performed in F_3BC_1. The variety Progress of interspecific origin (*G. hirsutum* L. × *G. barbadense* L.) was involved in the pedigree of the mother form line Number 37.

Very valuable for the cotton breeding were also the lines. Number 426 - $F_{11}BC_2$ (C_6 9736 × *G. thurbiri*) × line 413 × line T-073. This line exceeded the standard in seed cotton yield by 7.2% and equalized with it in fiber length and fiber lint percentage. Numbers 449 and 457 - both lines exceed the standard in seed cotton yield by 7.4% and 9.7%, respectively, in fiber length - by 1.2 mm and 0.5. They were inferior to the standard variety in fiber lint percentage. Both lines No. 449 and No. 457 were obtained by interspecific hybridization from the crossing of the allotetraploid *Gossypium thurberi* Tod. × *G. raimondii* Ulbr. with Darmi variety - *G. hirsitum* L. and backcrossing of the triple hybrid [(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × Darmi] with Darmi variety.

Number 346 and number 489 - these two lines combined longer fiber and higher yield than the standard variety. They were obtained by intraspecific hybridization: No.346 from the crossing of Natalia × Millennium (Greek variety); No.489 from the crossing of Chirpan-539 × line 713. The *G. barbadense* L. germplasm was included in the genotype of Natalia variety.

From these lines, two new candidate varieties - lines No.449 and No.535 were submitted for testing in the Exclusive Agency of Variety Testing, Seed Control and Approbation (EAVTSCA) in 2017.

According to EAVTSCA data, seed cotton yield of 2328 kg/ha was obtained from line 457 on average over two years, which was 13.0% above the average standard - 2060 kg/ha (average from the standard varieties Chirpan-539 - 2091 kg/ha and Avangard-264 - 2029 kg/ha) and seed cotton yield of 2026 kg/ha was obtained from line 535, which was 1.7% below the average standard. For line 457 the September yield of 1984 kg/ha was 16.0% higher than the average standard (1711 kg/ha). Line 457 in fiber yield - 859 kg/ha exceeded the average standard (759 kg/ha) by 13.2%, line 535 in fiber yield - 792 kg/ha exceeded it by 4.3% as a result of the higher lint percentage. Line 535 in fiber lint percentage - 39.0% exceeded the average standard (36.8%) by 1.8%.

Compared to the two standards (Chirpan-539 – standard for earliness and productivity and Avangard-264 – standard for fiber quality) both lines were distinguished by a higher SCI Index (consistency) of spinning - 126 and 124 (the average of the two standards was 116, 114 for Chirpan-539 and 118 for Avangard-264). Line 535 had lower micronaire of the fiber than both standards – 4.44 MIC, at 4.87 MIC for Chirpan-539 and 4.62 MIC for Avangard-264. Line 457 in fiber fineness – 4.61 MIC was equal to Avangard-264, but had finer fiber than Chirpan-539. Both lines had longer fiber than the two standards, by 1.0 mm and 1.1 mm, respectively, above Chirpan-539 and 0.3 mm and 0.4 mm above Avangard-264. Line 457 had better fiber uniformity - 83.2% compared to 81.2% for both standards, while line 535 (82.0%) was equal to the standards. Line 535 had higher fiber strength - 29.0 g/tex, at 27.8-27.9 g/tex for the standards. Line 457 was equal to the standards. Both lines had low content of short fibers – 8.1-8.8%, as in the standard varieties – 8.5-9.0 %.

Line 457 was approved by EAVTSCA as the new cotton variety Aida in 2020. Line 535 will continue its testing one year more. In 2018, lines 550 and 553 were included in the EAVTSCA system for testing as new candidate-varieties. Both lines confirmed their qualities in the state variety testing. Number 553 has the same origin as lines 449 and 457; it was obtained after backcrossing of the three-species hybrid [(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × Darmi] with Darmi variety.

A two-factor analysis of variance for 2014-2017 was performed on the traits - seed cotton yield, boll weight, fibre length and fibre lint percentage, of the 8 lines obtained through intraspecific and interspecific crosses. In the total variation of all traits studied, the years had the highest relative share: 64.50% for the seed cotton yield; 51.57% for the boll weight; 59.63% for the fiber lint percentage and 71.96% for the fiber length. For the seed cotton yield and boll weight, after years, the interaction of lines × years was important indicating that for these two traits the lines reacted specifically to environmental conditions (Table 2). In the period 2015-2017, the interaction of lines × years - 40.14% was of great importance for the seed cotton yield. The genotype of the lines -22.19% was also important for the size of the yield. Considering that seed cotton yield during this period was mainly determined by the genotype-environment interaction, the selection of the lines by seed cotton yield should be conducted in different environments (years). In terms of fiber lint percentage, the involvement of the lines was 17.38%, indicating that their genotype was also important for the formation of this trait. The genotype and the genotype-environment interaction had relatively equal shares in the total variation of the fiber length. The selection of lines by this fiber property should be conducted in years favorable for its formation, with precipitation in July, early August and not very high, stressful temperatures.

Table 2. Two-factor analysis of variance of the traits of the lines included in Competitive Variety Testing in 2014-2017

Sources of variation	Degree of freedom	Sum of squares	Correlation ratio Sum of squares, %	Dispersion	F-test
	Seed cotton yield, kg/ha (average for 4 years)				
Total	127	1117200	100.0	-	-
Replications	3	9742.5	0.87	3247.5	2.44
Variants	31	983897.5	88.07	31738.63	23.89+++
Lines-A	7	93917.5	8.4	13416.79	10.09+++
Years-B	3	721050	64.50	240350	180.90+++
Lines×years (A×B)	21	168930	15.12	8044.286	6.05+++
Errors	93	123560	11.06	1328.602	-
	Seed cotton yield, kg/ha (average for 3 years)				
Total	107	230970	100.0	-	-
Replications	3	5365	2.32	1788.333	1.78
Variants	26	147437.5	63.83	5670.673	5.66+++
Lines-A	8	51242.5	22.19	6405.313	3.39+++
Years-B	2	3482.5	1.51	1741.25	1.73
Lines×years (A×B)	16	92712.5	40.14	5794.531	5.78+++
Errors	78	78167.5	33.84	1002.147	-
	Boll weight, g				
Total	143	32.73926	100.00	-	-
Replications	3	0.101074	0.309	0.0337	0.365
Variants	35	22.95874	70.126	0.6559	7.115+++
Lines-A	8	2.329834	7.116	0.2912	3.159++
Years-B	3	16.88428	51.571	5.6281	61.052+++
Lines×years (A×B)	24	3.744629	11.437	0.1560	1.692++++
Errors	105	9.679443	29.565	0.0921	-
	Fiber lint percentage, %				
Total	143	693.625	100.00	-	-
Replications	3	0.281	4.05	0.9375	0.161
Variants	35	362.3906	91.17	18.0683	31.12+++
Lines-A	8	120.5625	17.38	15.07031	25.96+++
Years-B	3	413.625	59.63	137.875	237.51+++
Lines×years (A×B)	24	98.203	14.16	4.0917	7.04+++
Errors	105	60.953	8.79	0.581	-
	Fiber length, mm				
Total	143	283.2735	100.00	-	-
Replicattions	3	2.0625	0.73	0.6875	2.858+
Variants	35	255.9531	90.35	7.3129	30.40+++
Lines-A	8	28.21875	9.96	3.5273	14.664+++
Years-B	3	203.8438	71.96	67.9479	282.468+++
Lines×years (A×B)	24	23.89063	8.43	0.9954	4.138+++
Errors	105	25.25781	8.92	0.2405	-

+ - $p \leq 0.05$; ++ - $p \leq 0.01$; +++ - $p \leq 0.001$

For the average of three years (2016-2018) lines No.639 and No.641, included in the second competitive variety trial and tested for the third year, significantly exceeded the standard in seed cotton yield by 12.6% and 11.4%, in fiber length - by 1.6 mm and 0.9 mm and in fiber lint percentage - by 1.7% and 1.1%, respectively (Table 3). These two lines were also obtained by remote hybridization of *G. hirsutum* L. with the synthetic allotetraploid of the wild species *G. thurberi* × *G. raimondii* and saturating backcrosses of the triple hybrid.

Of the lines included in competitive variety trial for the second year (obtained by intraspecific hybridization from diallel and compound crosses), eight lines had significantly higher seed cotton yield than the standard Chirpan-539, five lines had longer fiber and five lines had higher lint percentage (Table 4).

Table 3. Agronomic qualities of lines in competitive variety trial in 2016-2018 (average for three years)

Line No.	Seed cotton yield, kg/ha	In % to Chirpan-539	Boll weight, g	Fiber length, mm	Fiber lint percentage, %
Chirpan-539*	1391	100.0	5.1	25.9	39.2
639	1567	112.6^{+++}	5.0	27.5^{+++}	40.9^{+++}
641	1549	111.4^{+++}	5.2^{+}	26.8^{+++}	40.3^{+++}
GD 5.0%	51	3.7	0.2	0.4	0.6
GD 1.0%	70	5.0	0.3	0.5	0.8
GD 0.1%	93	6.7	0.4	0.7	1.1
Accuracy	2.0		2.9	0.8	0.9

*Standard; + - p≤0.05; +++ - p≤0.001

Table 4. Agronomic qualities of lines in competitive variety trial for the 2nd year, obtained from diallel and double crosses in 2017-2018 (average for two years)

Line No.	Seed cotton yield, kg/ha	In % to chirpan-539	Boll weight, g	Fiber length, mm	Fiber lint percentage, %
Chirpan-539*	1405	100.0	5.3	25.5	40.7
		From diallel crosses			
668	1501	106.8^{++}	5.2	25.3	39.9
669	1464	104.2	5.1	25.4	40.4
671	1544	109.9^{+++}	5.5	26.3^{++}	38.7^{000}
672	1460	103.9	5.1	25.7	39.6^{0}
673	1464	104.2	5.2	26.6^{+++}	40.5
675	1501	106.8^{++}	5.5	25.9	40.2
678	1561	111.1^{+++}	5.5	27.0^{+++}	39.9
		From double crosses			
652	1432	101.9	5.2	25.2	41.6^{+}
654	1507	107.3^{++}	5.4	25.6	41.4
657	1423	101.3	5.6^{+}	26.1	41.7^{+}
661	1587	112.9^{+++}	5.0^{0}	25.5	42.8^{+++}
662	1519	108.1^{++}	5.0^{0}	27.0^{+++}	42.1^{++}
664	1488	105.9^{+}	5.0^{0}	26.3^{++}	42.2^{++}
GD 5.0%	72	5.1	0.3	0.6	0.9
GD 1.0%	96	6.8	0.4	0.8	1.2
GD 0.1%	124	8.8	0.5	1.1	1.6
Accuracy	2.5		2.6	1.2	1.1

*Standard; + - p≤0.05; ++ - p≤0.01; +++ - p≤0.001

On average over the two years the best were lines Number 662 - by 8.1% significantly higher seed cotton yield than the standard variety, 1.5 mm longer fiber and 1.4% higher fiber lint percentage, Number 664 - by 5.9% significantly higher seed cotton yield than the standard, 0.8 mm longer fiber and 1.5% higher fiber lint percentage. The two lines have the same origin, they were obtained through compound double crosses, from the crosses of F_1 (Barut × Dorina) × F_1 (Mytra × Helius).

Very valuable for breeding were lines Number 678 - by 11.1% significantly higher seed cotton yield, 1.5 mm longer fiber, but in fiber lint percentage it was inferior by 0.8% to the standard. This line was obtained from the remote hybridization of *G. hirsutum* L. with the wild diploid species *G. thurberi* Tod. - F_5BC_1 (C_6 9736 × *G. thurberi*) × Darmi variety, Number 661 - by 12.9% higher seed cotton yield and 2.1% higher fiber lint percentage, in the fiber length it was equal to the standard variety. Number 654 combined high productivity, seed cotton yield 7.3% above the standard and high fiber lint percentage - 41.4%, against 40.7% for the standard. Number 671 had high productivity, seed cotton yield 9.9% above the standard, and 0.8 mm longer fiber. Number 657 combined productivity, big boll weight and high fiber lint percentage - 41.7%, against 40.7% for the standard. Lines number 633 and 673 were insignificantly inferior to the standard in seed cotton yield by 5.9 and 4.2% respectively, having 0.8 mm longer fibre and in fibre lint percentage they were equal to the standard. Lines 668 and 675 in seed cotton yield exceeded the standard by 6.8% and were equal to it by fiber length and fiber lint percentage.

Conclusion

As a result of breeding for productivity and fiber quality, new cotton lines have been created combining seed cotton yield, fiber length and fiber lint percentage. Lines 550, 639, 641 obtained through remote hybridization appeared to be very promising. These three lines were distinguished by the best combination of productivity, fiber length and fiber lint percentage and by these three indicators they exceeded the standard variety Chirpan-539. A new cotton variety Aida (No.457) was approved, which in seed cotton yield and fiber

yield, and technological fiber properties exceeded the standard varieties Chirpan-539 and Avangard-264. Candidate variety 535 continued the state testing. Two new candidate varieties No.550 and No.553 were released. In the state variety testing they confirmed their qualities. The resulting lines, distinguished by one trait or by a complex of qualities, enriched the gene pool of Bulgarian cotton.

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