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The Cotton Breeding in Bulgaria

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Abstract

During 2011-2013 in comparative variety trial 11 new and two standard cotton varieties were tested. The varieties were obtained from two different targeted programs – for yield and for fiber quality with the application of different breeding methods. The results of the two-factor analysis (varieties-years) showed that the differences between the studied characters, except first picking, were significant, which means the varieties differed in them. Year conditions had the highest participation in the general variation of all characters (52.58 to 67.08%). The varieties Pearla-267 and Vicky had the highest seed cotton yield, exceeding the standard variety Chirpan-539 by 10.2 to 10.6%, average of three years. Best combination of length and lint percentage of the fiber was found for the varieties Darmi, Vega and Boyana. The studies are still focused on: creation of source material with diverse germplasm; maintenance and use of the genetic resources; heterosis based on male sterility; application of biotechnologies; drought resistance with reference to the selection of tolerant genotypes; resistance to Verticierum dahlia Kleb and seed production of regional varieties.

Key words: Cotton, *G. hirsutum L.*, breeding, varieties, yield, fiber quality

Introduction

In Bulgaria, the breeding work with cotton was initiated by Malkov in 1901 in the Experimental Station in Sadovo, Plovdiv district. In 1925, selection and genetic improvement of cotton began at the Institute in Chirpan, Stara Zagora district, and in 1953 in the Experimental Station in Pavlikeni (Northern Bulgaria) and in the Higher Agricultural Institute in Plovdiv, now Agrarian University.

As a result of successful selection many cotton varieties were developed and implemented in production of which Nos. 38, 2362, 3279, 4521, Pavlikeni-73 and Chirpan-433 were particularly valuable (Milkovski et al., 1972). Variety No. 38 was obtained by selection in local population, and the other varieties were created through intervarietal hybridization.

Koynov (1981) reported the first Bulgarian long-fiber cotton varieties produced through interspecific hybridization of *G. hirsutum L.* × *G. barbadense L.* These varieties were later mature and did not found in practice. Bulgaria is located on the northern border of the cotton culture distribution and the earliness of varieties is of crucial importance for the breeding.

The period 1980-2000 was particularly fruitful for the selection of cotton, when the varieties Beli izvor (432), Garant (996) Ogosta (644) (Bojinov and Dimitrova, 1981; 1989), Chirpan-539, Chirpan-603 (Bojinov et al., 1996) and Avangard-264 (Koynov and Stoilova, 1996) were created and introduced in the production. Breeding work with cotton during this period was concentrated entirely in the Cotton and Durum Wheat Institute, Chirpan, now Field Crops Institute.

The variety Beli izvor possessed a complex of positive qualities and high adaptive capacity and stood longest in production, since 1981 until 2003 was the main variety in the country. Chirpan-539 and Avangard-264 varieties were approved in 1994, as the first was obtained trough intraspecific hybridization and the second one was created after the interspecific *G. hirsutum L.* × *G. barbadense L.* hybridization. The variety Chirpan-539 is very early, highly productive, with high lint percentage of the fiber, while the variety Avangard-264 has a higher quality of the fiber. The two varieties are now national standards, Chirpan-539 for earliness and productivity, Avangard-264 for the fiber quality.

In 2003, the Expert Committee on cotton at the Executive Agency for Variety trials, Approbation and Seed control approved three new cotton varieties - Beli Iskar, Beli Lom and Vega. The average fiber lint percentage of Beli Lom was 39.8%, by 1.8% more than Chirpan-603 (standard), and was the variety with the highest lint

percentage among the Bulgarian varieties (Bozhinov and Bozhinov, 2004).

Recently, for a very short time a number of new cotton varieties possessing high genetic potential for yield or improved fiber quality have been created (Stoilova and Valkova, 2008; Stoilova et al., 2010).

The aim of this study was to examine the productive capacity of the new cotton varieties created in the Field Crops Institute, Chirpan in recent years.

Material and Methods

During the period 2011-2013 in comparative variety trial 11 new Bulgarian cotton varieties - Helius, Boyana, Vicky, Perla-267, Vega, Colorit, Darmi, Natalia, Rumi, Dorina, Nellina, and two standards Chirpan-539 (for earliness) and Avanguard-264 (for fiber quality) were included. The trial was set by a block method design, in four replications and harvesting plot of 20 m^2. September and total seed cotton yields, boll weight, length (by "butterfly") and lint percentage of the fiber were accounted. Data were statistically analyzed by applying of ANOVA 123 statistical program.

Meteorological characteristics in relation to growth and development of cotton during the period of study

In 2011 and 2013, during the period of germination, the rainfall security in May was lower than the average of many-year period **(Table 1).** By this reason, the germination was prolonged and irregular (storey) crops were produced - plants grown in optimal time (around 10-25%), plants grown after rainfall in early June and plants germinated between June 25 to July 5 (massively, 2013).

Table 1. Meteorological characteristics of the region at the Field Cops Institute, Chirpan during the period April-September 2011-2013 compared with many years values

Years	Months						Σ IV-IX	Σ VI-VIII	Σ V-IX
	IV	V	VI	VII	VIII	IX			
Temperature sum *Σ t ^0C*									
1928-2007	343	519	622	720	711	561	3476	2053	3133
2011	535	538	645	772	743	558	3791	2160	3256
±	192	19	23	52	32	-3	315	107	123
2012	412	519	688	830	787	623	3859	2305	3447
±	69	-	66	110	76	62	383	252	314
2013	416	604	639	710	785	581	3735	2134	3319
	73	85	17	-10	74	20	259	81	186
Rainfall - *mm*									
1928-2007	45	63	65	52	41	34	300	158	255
2011	46	46	31	24	58	50	255	113	209
±	1	-17	-34	-28	17	16	-45	-45	-46
2012	14	128	16	7	10	10	185	33	171
±	-31	65	-49	-45	-31	-24	-115	-125	-84
2013	41	14	61	91	1	10	218	153	177
±	-4	-49	-4	39	-40	-24	-82	-5	-78

The vegetation period of cotton in 2011 was accompanied by a prolonged summer drought and higher temperatures. Thus, in June, July and August rainfall was total 111 mm against 158 mm for a many year period, by 29.7% less, as in July only 24 mm rainfall were recorded. The temperature sum for the same period was 11.6% more. HTC (Hydro-thermal Coefficient by Selyaninov) was almost twice lower than the normal - 0.32 against 0.77.

In 2012, for the months of June, July and August total rainfall was only 33 mm against 158 mm for a many year period, by 79.1% less, as in July only 7 mm rainfall was marked. The temperature sum for the same period was 11.2% more and HTC was far below normal, only 0.14 versus 0.77. Rainfall in August (10 mm) and September (10 mm) have helped to retain the bolls

on the upper floors of the cotton bushes in the later genotypes, but were not harvested because of the earlier harvest of the trial.

The vegetation period of cotton in 2013 passed at relatively favorable conditions and higher temperatures. So, for the months of August and September, the temperature sum was by 94 °C higher than the average of many years, while rainfall during the same period was 64 mm versus 75 mm.

Harvesting of cotton in 2011 was carried out in adverse temperature and humidity conditions - 16 mm more rainfall in September, almost double in comparison with the perennial value.

Results and Discussion

The results of the two-factor analysis (varieties × years) showed that the differences were significant for the boll weigh, lint percentage and length of the fiber, which means that the varieties differed in them and the variability was well expressed **(Table 2).** The differences were slightly significant for the total seed cotton yield and insignificant for the first harvest. Year conditions had the highest significant participation in the general variation of all characters (52.58 to 67.08%). Interaction variety × year was significant for the fiber length and lint percentage, less significant for the total seed cotton yield and insignificant for the boll weight. For the first three characters it is necessary to evaluate the varietals' stability. Strong influence of the years in the general variability of the characters and genotype-environment interaction indicates that the selection should be aimed at creating varieties with wide ecological plasticity or high stability.

Table 2. Results of two-factor ANOVA of the characters of the varieties included in the study in 2011-2013 (average for 3 years)

Source of variation	Degrees of freedom	Correlation terms	Dispersion
		First picking	
Varieties - A	12	7.13	907.58
Years - B	2	27.7	2140.63^{+++}
Varieties × years (A × B)	24	16.49	1048.49^{+}
Error - E	114	41.46	555.09
		Seed Cotton Yield	
Varieties - A	12	3,63	744.96^{+}
Years - B	2	67.08	82525.75^{+++}
Varieties × years (A × B)	24	8,56	877.58^{++}
Error - E	114	18,51	399.51
		Boll weight	
Varieties - A	12	9,02	0.229^{+++}
Years - B	2	57.02	8.709^{+++}
Varieties × years (A × B)	24	7.71	0.098
Error - E	114	25.78	0.069
		Lint percentage	
Varieties - A	12	37.67	15.61^{+++}
Years - B	2	52.58	130.69^{+++}
Varieties × years (A × B)	24	4.04	0.837^{+++}
Error - E	114	5.52	0.241
		Fiber length	
Varieties - A	12	10.37	2.01^{+++}
Years - B	2	62.67	72.90^{+++}
Varieties × years (A × B)	24	17.41	1.69^{+++}
Error - E	114	9.52	0.19

The varieties Perla-267 and Vicky had the highest seed cotton yield and exceeded the standard Chirpan-539 by 10.2 to 10.6%, average of three years **(Table 3).** Colorit, Helius, Nellina and Rumi varieties exceeded the standard insignificantly by 6.5 to 9.1%. Natalia, Darmi and Dorina varieties surpassed it slightly by 2.1 to 3.7%. Vega variety was aligned with the standard (99.0%), Boyana slightly and insignificantly (95.4%) felt behind it. The highest index of earliness (1st picking in percent to total seed cotton yield) was found for the standard and Perla-267 variety, and the lowest – for Vega variety.

Table 3. Agronomic characters of the varieties included in the study in 2011-2013 (average for 3 years)

Variety	Seed cotton yield *kg/ha*	In percent to Chirpan-539	1st picking *kg/ha*	In percent to total yield	Boll weight *g*	Fiber length *mm*	Lint percentage *%*
Chirpan-539	1655	100	1418	85.7	5.3	26.4	39.9
Avangard-264	1743	105.3	1476	84.7	5.4	27.3^{+++}	36.7
Perla-267	1822	110.2^{+}	1567	86	5.4	27.4^{+++}	36.7
Natalia	1699	102.7	1430	84.2	5.3	26.8^{+}	37.2
Darmi	1717	103.7	1446	84.2	5.3	27.2^{+++}	38
Colorit	1806	109.1	1482	82.1	5.3	27.0^{++}	37.1
Vega	1638	99	1283	78.3	5.2	27.1^{++}	38
Dorina	168,9	102.1	1401	82.9	5.2	27.3^{+++}	39.7
Nellina	1789	108.1	1506	84.2	5	26.7	39.9
Rumi	1762	106.5	1493	84.7	5.2	27.2^{+++}	37.5
Helius	1805	109.1	1456	80.7	5.3	26.2	37.5
Boyana	1579	95.4	1280	81.1	5.2	27.1^{++}	38
Vicki	1831	110.6^{+}	1551	84.7	5	26.3	38.2
GD 5.0 %	162	9.8	190	-	0.2	0.4	0.4
GD 1.0 %	214	12.9	252	-	0.3	0.5	0.5
GD 0.1 %	276	16.7	325	-	0.4	0.6	0.7

Nellina and Vicky varieties had less boll weight than the standard variety, while the others were equal with it. Qualitative varieties had 26.8-27.4 mm fiber length, the standard variety – 26.4 mm. Helius and Vicky varieties in fiber length (26.2-26.3 mm) were aligned with the standard. As for the fiber lint percentage diversity among the varieties was larger. Chirpan-539, Dorina and Nellina varieties had the highest lint percentage of the fiber - 39.7-39.9%. All other varieties had lower fiber lint percentage. Among them, Darmi, Vega, Boyana and Vicky had higher values of this character (38.0 to 38.2%). Most of the qualitative varieties had low fiber lint percentage - 37.2-37.5%. Good combination of length and lint percentage of the fiber was found for the varieties Darmi, Vega and Boyana.

The varieties were obtained from two different targeted programs - for productivity and for fiber quality, by the application of different breeding methods. Helius variety was created by experimental mutagenesis (Valkova, 2009), Boyana and Vicky varieties - by intervarietal hybridization (Valkova, Bozhinov, 2010), Pearla-267, Vega, Darmi, Colorit, Natalia, Rumi and Nellina - by the crossing of introgress *G. hirsutum L. × G. barbadense L.* lines with the *G. hirsutum L.* varieties (Stoilova, Saldzhiev, 2005; 2008a; 2008b; 2010; Stoilova, Meluca, 2013). Helius and Vicki varieties are a new stage in the selection of earliness and productivity, while the others are new achievements in the fiber quality selection. The results obtained from the state testing of all varieties, their origins and descriptions are

presented in previous publications (Stoilova, Valkova, 2008; Stoilova et al. 2010).

Plovdiv, Philippopolis and Denitza varieties approved in 2012-2013 are the newest achievements in the selection of earliness and productivity. In 2014, in the system of Executive Agency for Variety trials, Approbation and Seed control three new candidate-varieties of cotton are tested. Through hybridization within the *G. hirsutum L.* species new lines are obtained realizing yield significantly higher than the standard. Line No. 595 stands out for its very high yield and long fiber. By applying of experimental mutagenesis lines (646, 657, 653, 639 and 655) that exceed Chirpan-539 in all valuable for selection characters are created. As a result of fiber quality selection lines with germplasm of the *G. barbadense L.* species (210, 262, 265, 339 and 346) combining yield, length and lint percentage of the fiber are realized. By distant hybridization of *G. hirsutum L.* with the wild diploid species *G. sturtii F. Mull, G. thurberi Tod., G. davidsonii Kell., G. raimondii U.* lines (429 and 457) having higher productivity by 16.3 to 22.5% over the standard and by 2.3 mm longer fiber (449) are created (Summary Report 2011-2013). All lines are tested in comparative variety trials.

Creation of early varieties possessing high genetic potential for yield over 4500-5000 kg/ha and selection of varieties of high fiber quality are priority directions in the cotton breeding programs.

Breeding studies are focused still on: creation of source material with diverse germplasm; maintenance and use of genetic resources; heterosis based on male sterility; application of biotechnologies; drought resistance with reference to the selection of tolerant genotypes; resistance to Verticillium dahliae Kleb. and seed production of regional varieties

Genetic studies are focused on: general and specific combining ability of lines and varieties and inheritance of most important agronomic traits; reaction of genotypes to the year conditions (genotype-environment interaction, stability and plasticity of varieties); evaluation of genotypic diversity through different analyses (including cluster analysis, component analysis, and etc.) and optimizing the selection process by application of various statistical models.

Hybrid plants BC_1F_1 were obtained after backcrossing of the amphidiploids' *Gossypium hirsutum* × *G. sturtianum* 2[$(AD)_1C_1$] (2n=78) with the species G. *hirsutum L.* $2(AD)_1$ (variety Chirpan-539) by applying the method of embryoculture. To prevent the early flower buds shedding, the hybrid plants were treated with a solution of NAA (50mg l-1), naphthalene acetic acid + GA_3 (100mg l-1), gibberellin acid, immediately after pollination. Hormonal action provided the retention of the hybrid fruits until the embryos reached development for the introduction in culture.

After self-pollination of the BC_1F_1 plants, the formed fruits were treated with growth regulators and left on the mother plant. From the resulting 40 seeds only one of them germinated and grew to adult plant - second generation of the backcross one (BC_1F_2).

The plants of the first backcross generation (amphidiploids' × Chirpan-539) BC_1F_1 were pollinated with pollen from the variety Chirpan-539. Of 42 crosses, which were realized, 7 viable embryos in various stages of development were isolated **(Table. 4).** Part of differentiated embryos developed to plants with well developed root and germ, while the others failed at different stages of their development because of reduced viability **(Fig. 1).**

Table 4. Regeneration of plants after embryos culture

Hybrid combinations	Pollinated flowers Number	Introduced into the environment embryos Number	Regenerated plants Number
(amphidiploid×Chirpan-539) BC_1F_1-harvest 2007 × Chirpan-539	23	5	2
(amphidiploid×Chirpan-539) BC_1F_1-harvest 2008 × Chirpan-539	19	2	1

Cytological observations on fertility of pollen in BC_1 and BC_1F_2 generations showed a high sterility of the anthers grains. Pollen fertility of BC_1 plants was very low 20.5% and similar was of BC_1F_2 plants - 16.7%. Uncolored pollen grains of irregular shape and size smaller than normal ones were observed. The different levels of ploidy and differences in the genomic composition of the two species have resulted to disorders in the meiotic

process and the formation of generative cells and in the more advanced generations of hybrid plants.

The assessment on the cotton varieties sustainability showed that there were no genotypes immune and resistant to Verticillium dahliae Kleb. Intensive development of Verticillium dahliae in cotton in our country is favored by the infection at an earlier stage, and rainfall in July is of decisive importance (Nedyalkova, 2009).

It was found that of the examined parameters of the chlorophyll fluorescence the maximum photochemical activity of photosystem 2 (Fv/Fm) and the quantum yield of the electron transport (Y) were most informative for the state of the photosynthetic apparatus of the plants (Inamullah and Isoda, 2005).

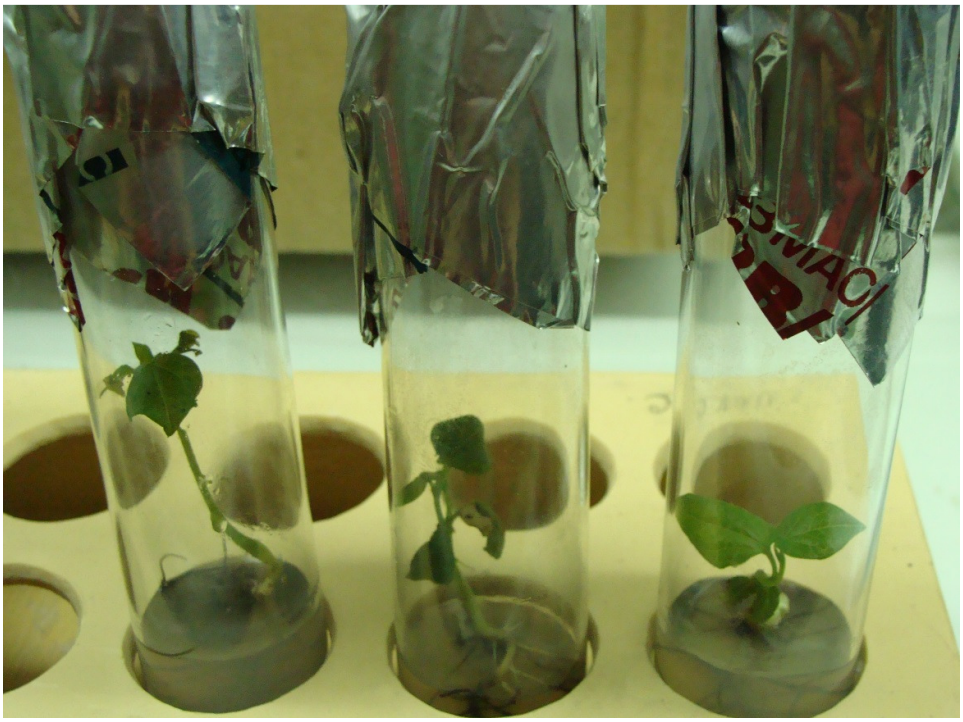

Fig. 1. Regenerated plants from the crosses (amphidiploid × Chirpan-539) BC_1 × Chirpan-539

Bulgarian cotton varieties had a relatively high tolerance to soil drought. Grown without irrigation, where a significant reduction in the soil moisture in the productive topsoil (50 cm) was seen, plants of all tested varieties had relatively good photosynthetic activity, based on the parameters of chlorophyll fluorescence. Potential photochemical activity of FC2 (Fv/Fm) was in the norm, typical of healthy plants and the actual quantum yield (Y) was high enough for plants grown under dry conditions (Koleva, Vasilev, 2012).

Cotton production in the country is developed under conditions of limited temperature resources and unstable rainfall security, and economic productivity of cotton is highly dependent upon agro-meteorological conditions during the vegetation period. It was found that the positive correlation of seed cotton yield with rainfall was stronger during July and August. Strong drought and high air temperatures during this period determine the high values of evapotranspiration. Without irrigation rainfall during this period to the greatest extent determine the magnitude of yield (Stoilova, 2012a). Critical periods of dependency of lint percentage and fiber length from the rainfall are 11 July to 10 August, for the late varieties such as Avangard-264 are 11 July to 20 August and from the temperature - 21 July to 10 August (Stoilova, 2012 a; 2012 b). The strong dependence of seed cotton yield, length and lint percentage of the fiber from the rainfall in July and August, period of summer drought, determines the selection of varieties tolerant to drought and high (stress) temperatures.

Fat content of Bulgarian cotton varieties bred in recent years, ranges from 17.13 to 25.97%. Trakia, Avangard-264, Vega and Perla varieties distinguished by higher oil content, Chirpan-539, Helius and Darmi have lower oil and higher protein content. If the cotton area in the country be restored of the energy sector more than 6000 tons oil for biodiesel could provide (Saldzhieva et al., 2009).

In the Field Crops Institute, Chirpan seed production is organized of both standard varieties Chirpan-539 and Avangard-264 and of two new cotton varieties - Helius and Darmi **(Fig. 2, 3).**

Fig. 2. Comparative test of 2nd year progenies of Helius and Darmi varieties

Fig. 3. Basic seedlings of Helius and Darmi varieties

Conclusion

In recent years, as a result of successful selection, a large diversity of varieties has been created, which is a good prerequisite for the development of cotton production in the country.

Bulgarian cotton varieties have realized yields of 1655 to 1831 kg/ha, average of three years. Perla-267 and Vicky varieties have emerged as the most highly productive, by 10.2-10.6% over the standard Chirpan-539.

Good combination of length and lint percentage of the fiber was found for the varieties Dorina, Darmi, Vega and Boyana. Chirpan-539, Dorina and Nellina varieties had the highest fiber lint percentage (39.7-39.9%).

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