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Specialized trait collections for the different selection directions in cotton. II. Specialized trait collections based on exceptional characteristics for ecological cotton production and heterosis selection

Neli Valkova, Valentina Dimitrova, Minka Koleva* and Spasimira Nedyalkova

Agricultural Academy, Field Crops Institute – Chirpan, 6200 Chirpan, Bulgaria
**Corresponding author:* m_koleva2006@abv.bg

Abstract

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Specialized trait collections based on exceptional characteristics for ecological cotton production and heterosis selection have been created: specialized collection based on naturally colored cotton (brown and green); specialized collection based on naked seed genotypes of cotton; specialized collection based on forms with male sterility. Organization of the relevant collections was in accordance with the requirements of selection process for a complex assessment of biological and economic qualities, adaptability to different environmental conditions, resistance to diseases and enemies. Created collections will facilitate the use of available genetic resources in cotton breeding to solve some existing ecological problems in the production and heterosis selection, to create varieties with high economic and ecological effects, and high-yielding hybrids based on male sterility. On the other hand, with them, commercial and other available germplasm will be preserved and the hereditary basis of cotton selection in the respective directions will be enriched and expanded.

Keywords: colored cotton; cytoplasmic male sterility; genetic resources; naked seed

Introduction

There are unsolved technological, economic and environmental problems related to the growing cotton technology and preparation of seeds for sowing. Synthetic dyes are used to dye cotton yarns and fabrics, which cause allergies. During the preparation of cotton seeds for sowing, chemical delinting (fuzz removal) is performed using sulfuric acid (negative ecological effect and high economic costs), and abundant washing of seed mass with water and drying of seeds (additional consumption of water and energy). Production of hybrid cotton requires labour intensive hand emasculation. Economic and ecological problems in cotton production increasingly necessitate creation of a new gene pool and a new generation of varieties, which, in sync with the intensive factors, to provide high biological, economic and ecological effects.

Development and introduction of naturally colored fiber cotton varieties and naked seeded varieties as well as hybrids based on cytoplasmic male sterility can solve many of the problems in cotton production.

Many industrialists and researchers have turned their attention to the advantages, disadvantages and prospects of colored cotton (Gülümser, 2016; Keshamma et al., 2021; Muqaddim, 2022).

Colored cotton is found among cultivated and wild species of the genus *Gossypium* L. Large range of naturally colored cottons in brown, green, blue, pink, red, and also black, have been reported in the specialized literature. Brown and green cottons are the most widely cultivated with the brown ones being more widespread, and there colors are more stable than green cottons (Singh et al., TB No: 4; Hidayat et al., 2020).

Colored cotton is grown in many countries in small farms in Peru, India, Brazil, USA, China, Israel, Indonesia, Russia and others. Bronesia 1, Bronesia 2 and Bronesia 3 were the first colored cotton varieties released in Indonesia (Hidayat et al., 2020).

Usually, colored cottons are more primitive with lower productivity and their fiber is shorter, coarser, with less strength and lower lint index. Many researchers consider that the coloured cotton fiber technological qualities could be improved through effective selection.

Work on the creation of colored cotton is carried out in a number of countries such as the USA, India, Brazil, China, Pakistan, Uzbekistan, Turkey, etc. In Australia, through genetic modifications and transformations, colored cotton has been created in black and other deep, dark colors (Keshamma et al., 2021).

Many researchers have studied the mechanisms of male sterility to solve the problems of manual labor and make hybrid seed production cheaper (Wu et al., 2011; Li et al., 2018; Nie et al., 2018; Ma et al., 2021; Zhang et al., 2021; You et al., 2022).

Depending on the factors causing sterility there are genetic male sterility, functional male sterility and induced male sterility (Kaul, 1998). There are three kinds of genetic sterility – genetic (nuclear, genetic) male sterility, cytoplasmic and cytoplasmic-genetic (Lecture 7, 2021). All three male sterility types are found in cotton (Singh et al., TB No: 24). GMS is widely used for hybrid seed production in India in diploid and tetraploid cotton (Raja et al., 2018).

Sixteen different genes in tetraploid cottons (13 in *G. hirsutum* L. and 3 in *G. barbadense* L.) and two in *G. arboreum* L. have been identified for genetic male sterility (Singh et al., TB No: 24). Morales et al. (2022) reported 23 new genes for male sterility in upland cotton. Cytoplasms of the diploid species *G. arboretum* L. (cultivated) *G. anomalum*, *G. trilobum* Skov. (Meyer, 1969; Stewart, 1999), *G. harknessii Brandeg*, designated D_2 (Meyer, 1973; 1975), *G. aridum* Skov., *G. sturtianum Willis* (wild) were found to have sterilizing effect, when they interact with the nuclear genes of *G. hirsutum* L. cause male sterility (Singh et al., TB No: 24).

Temperature, photoperiod and other environmental factors could cause male sterility. High temperatures induced male sterility in cotton (Khan et al., 2020; Zhang et al., 2020; Li et al., 2022). Zhang et al. (2023) reported a photosensitive genetic male sterile mutant in upland cotton.

In order to obtain high-quality and high-yielding hybrids based on male sterility (GMS and CMS) great attention was paid to studying the degree of heterosis, combining ability and inheritance of economically valuable traits (Nirania et al., 2004; Tuteja et al., 2004; Xuede et al., 2004; Tuteja et al., 2005; Singh, 2006; Stoilova, 2008; Solanke et al., 2015).

Ahmad et al. (2014) studied interrelationships between different characters in upland cotton, and concluded that traits such as shape and color of flowers and bolls are very important as markers for yield contributing traits and fiber quality and revealed positive correlations between round boll shape and number of sympodial branches and boll weight; dark green boll color and plant height, number of bolls, boll weight, number of locals and fiber strength; bell flower shape with number of bolls, boll weight, fiber strength, length and uniformity; tubular flower shape with fineness. Morphological characters can also be useful indicators for heterosis selection with cotton.

Potential for application of biotechnology such as identification and use of molecular markers (Raja et al., 2018), gene editing and mutation induction (Zhang et al., 2023), somatic hybridization to transfer GMS from wild species (Garcia et al., 2019) is great. There have been many reports regarding genetic and QTL modeling (Han et al., 2022), transcriptomic and metabolomic studies to identify genes associated with male sterility in cotton (Kong et al., 2017; Li et al., 2019; 2021; You et al., 2022).

The aim of this study was to create specialized trait collections according to selection directions and their importance as source material for the cotton breeding, based on exceptional characteristics for ecological cotton production and heterosis selection, and with a view to conserving the available germplasm and facilitating its use.

Material and Methods

On the basis of available genetic resources and new achievements in the selection of ecological cotton forms, at the Institute of Field Crops in Chirpan it was organized the creation of a specialized gene pool, including trait collections with specialized purpose. The collections were founded on exceptional characteristics for ecological cotton production and heterosis: specialized collection based on coloured cotton genotypes (brown and green); specialized collection based on cotton genotypes with naked seed; specialized collection based on cytoplasmic male sterility cotton forms to be used in heterosis selection. Organization of the relevant collections was in accordance with the requirements of selection process for a complex evaluation of biological and economic qualities, adaptability to the specific conditions, resistance to diseases and enemies. Seed cotton yield (kg ha^{-1}), lint percentage and fiber length (mm) data are presented only for some samples – new varieties and lines with exceptional characteristics included in the trait collections for ecological cotton production and heterosis selection.

Results and Discussion

Specialized collection based on coloured cotton genotypes (brown and green)

Given the EU criteria for ecologically clean production the cultivation of colored cotton in our country can prove to be very profitable. For our country, selection of colored cotton is new and not so intensively conducted. Forms with brown and green fibers have been obtained (Stoilova et al., 2009; 2011). In 2010, the first brown cotton variety Isabell was approved, marking the beginning of a new generation of varieties, with naturally colored fibers (Stoilova et al., 2010).

The purpose of creating a colored cotton collection was to develop naturally colored brown and green varieties, with high earliness and productivity.

Parameters: Vegetation period 110–115 days, fiber length 24.0–26.0 mm, lint percentage over 34–36% and 1st fruit branch setting over 15 cm.

At this stage the collection includes brown cotton varieties Isabell, Egea and Nike, and brown and green lines and forms. The created brown varieties Isabell, Nike and Egea meet the set parameters.

Isabell variety was obtained from the crossing of selection lines No. 433 (brown) × No. 396 (white). It is characterized by good productivity and early maturity and in seed cotton yield is equal to the white standard cultivar Chirpan-539, inferior to it in lint percentage and lint yield.

In the IASAS network (2006–2008) in seed cotton yield of 1795 kg ha^{-1}, average for the test period, it was equal to the standard cultivars Chirpan-539 and Avangard-264 for white cotton. In lint yield and lint percentage it was inferior to them. It was distinguished by better earliness than the two standard cultivars and in September harvest (1603 kg ha^{-1}) surpassed Chirpan-539 by 4.4%, Avangard-264 – by 5.3%. It also had higher maturity index – 89.3% compared to 84.2% for Chirpan-539 and 83.9% for Avangard-264. Izabell variety in fiber length (23.35 mm modal and 27.0 mm staple) was inferior to the white standard cultivars, but in terms of other technological properties was equal or very slightly inferior to them.

Egea and Nike varieties are the next achievements in the colored cotton breeding in our country. The two varieties were approved in 2017. Both varieties are natural hybrids, Egea was selected from the cross Avangard-264 × Eva (Greek cultivar) and Nike – from the cross Chirpan-539 × line 40. The two varieties are characterized by good productivity, early maturity, suitability for mechanized harvesting and their fiber is longer and finer than that of Isabell variety.

Egea and Nike varieties, tested in the IASAS system (2013, 2015–2016), in earliness and seed cotton yield were equal to Isabell variety and approached the standard cultivars for white cotton. Average over the three years, seed cotton yield was 1921 kg ha^{-1} for Egeia variety and 1917 kg ha^{-1} for Nike variety, compared to 1946 kg ha^{-1} for the cultivar Chirpan-539 and 1933 kg ha^{-1} for the cultivar Avangard-264. In lint yield and lint percentage both varieties were equal to Isabell variety (IASAS 2013, 2015 and 2016).

Both varieties had longer fiber than Isabelle variety (Table 1). Egea and Nike varieties in modal (23.32 mm and 22.51 mm) and staple (26.8 mm and 25.63 mm) fiber length surpassed Isabell variety by 1.35 mm and 0.54 mm, and 1.57 mm and 0.37 mm, respectively. In Upper Half Mean Length (UHML) (23.51 mm and 23.60 mm), they surpassed it by 1.13 mm and 1.22 mm. Egea in fiber length was only 0.6–0.8 mm behind the white cotton standards. The both varieties were inferior to the white cotton standards in fiber strength, while in other technological fiber properties such as uniformity in length, elongation and percentage of maturity they were close to or equal to them.

In 2023, the three brown varieties and the latest white cotton varieties Bulgarian selection were included in a va-

Table 1. Fiber length of Egea and Nike varieties according to the IASAS data

Fiber properties	Variety					
	Average standard	Chirpan-539	Avangard-264	Isabell	Egea	Nike
2013–2015						
Staple length, mm	27.49	27.62	27.38	25.26	26.83	25.63
Modal length, mm	23.83	23.79	23.85	21.97	23.32	22.51
Upper Half Mean Length (UHML), mm	25.61	24.56	26.66	22.38	23.51	23.60
Fiber strength (Str), g tex^{-1}	27.3	26.8	29.1	24.3	23.5	23.8

Source: Authors' own elaboration

riety trial by the block method in four replications and a harvest plot of 20.52 m^2, and width between rows 95 cm. In the conditions of this year, the white cotton varieties Pirin, Krystal, Perun and Tiara produced the highest seed cotton yields of 1364–1437 kg ha^{-1}, Pirin variety was the most productive. (Table 2).

Isabell, Egea and Nike varieties have realized seed cotton yield of 1401–1558 kg ha^{-1} and the most productive was Egea variety. Nike variety in seed cotton yield was equal to Isabell variety, while Egea variety surpassed it by 11.2%. Isabell and Nike varieties in seed cotton yield were close to Pirin variety, while Egea variety surpassed it by 8.4 %.

Brown varieties had significantly lower lint percentage (33.6–36.0%) and shorter fiber length (22.4–24.1 mm) than white cotton varieties, typical for colored cotton. Egea and Nike varieties had longer fiber length (24.0–24.1 mm) than that of Isabell variety (22.4 mm), surpassing it by 1.6–1.7 mm.

Results for economic and technological fiber qualities of created brown cotton varieties are consistent with those reported by other authors in previous studies (Geng et al., 1998; Leonard et al., 1999; Du et al., 2000), that colored cotton is characterized by lower productivity and worse fiber quality than white cotton. According to the same authors, fiber technological qualities of coloured cotton could be improved by effective selection, which is confirmed with the creation of Egea and Nike varieties, both with longer fiber in different environments.

Lines No. 201 and No. 202, with light brown, and 221 and 222, with dark brown (rusty brown) fibers were also included in the collection. In a competitive variety trial line 201 was more productive than Isabell variety, had higher boll weight, longer fiber and slightly higher lint percentage (Table 3).

As a result of selection a number of brown lines have been obtained, which are in various stages of testing. Among them, there were genotypes very valuable for colored cotton breeding, with high productivity or longer fiber (Table 4).

Many of green (brownish green) cotton lines included in this collection had fiber length of 24.3–26.1 mm, compared to 26.5 mm for the standard cultivar white cotton Chirpan-539 (Table 5). Lint percentage was very low (27.0–31.9%). Their productivity was also low, as a result of delay in maturity. These lines, however, are very valuable genetic material for developing new cotton varieties with naturally colored green fiber.

Table 2. Comparative assessment of economic qualities of brown and white cotton varieties included in variety trial in 2023

Variety	Seed cotton yield (kg ha^{-1})	In % to Chirpan-539	Boll weight (g)	Lint percentage (%)	Fiberlength (mm)
Izabell	1401	125.0	5.1	36.0	22.4
Egea	1558	139.0	4.8	33.6	24.1
Nike	1416	126.3	5.1	35.5	24.4
Aida	1328	118.5	5.0	37.0	27.2
Pirin	1437	128.2	5.0	37.8	26.3
Perun	1364	121.7	4.9	38.6	26.6
Tiara	1366	121.9	5.2	38.7	27.3
Melani	1231	109.8	5.1	37.7	26.7
Kristal	1382	123.3	5.2	37.5	26.5
L.S.D. 5.0%	71	6.3	0.3	0.6	0.6
L.S.D. 1.0%	95	8.5	0.4	0.9	0.9
L.S.D. 0.1%	124	11.1	0.5	1.2	1.1

Source: Authors' own elaboration

Table 3. Test results of brown cotton lines in a competitive variety trial

Line No.	Seed cotton yield (kg ha^{-1})	In % to Isabell	Boll weight (g)	Fiberlength (mm)	Lint percentage (%)
Isabell	1221	100.0	4.6	22.6	34.8
201 Light brown	1413	115.7	4.8	24.5	35.5
202 Light brown	1263	103.4	4.6	22.3	35.8
221 Rusty brown	1259	103.1	4.4	23.4	34.4
222 Rusty brown	1189	97.4	4.7	22.3	37.1

Source: Authors' own elaboration

Table 4. Economic qualities of brown cotton lines in 2018

Line No.	Seed cotton yield (kg ha^{-1})	In % to Chirpan-539	Boll weight (g)	Fiber length (mm)	Lintpercentage (%)
Chirpan-539	1177	100.0	5.9	26.3	40.8
459	1228	104.3	5.8	25.7	32.0^{000}
460	1275	108.3	5.4^{0}	24.1^{000}	31.2^{000}
462	1784	151.6^{***}	5.3^{00}	24.0^{000}	36.3^{000}
463	1278	108.6	5.8	25.5^{0}	34.3^{000}
465	1273	108.2	5.1^{000}	25.1^{00}	33.9^{000}
469	1119	95.1	5.1^{000}	23.7^{000}	30.4^{000}
470	1325	112.5	5.3^{00}	23.5^{000}	32.3^{000}
471	1378	117.0^{**}	5.6	22.7^{000}	34.4^{000}
564	1673	142.1^{***}	4.5^{000}	24.5^{000}	32.00^{000}
594	1079	91.7	6.2	25.5^{0}	36.8^{000}
L.S.D. 5.0%	178	15.1	0.4	0.8	1.9
L.S.D. 1.0%	242	20.6	0.6	1.1	2.6
L.S.D. 0.1%	327	27.8	0.8	1.5	3.5

Source: Authors' own elaboration

In some years yields of colored cotton lines was low not only due to severe drought during the flowering and fruiting period, but also due to cross-pollination and genetic contamination of both colored and white cotton. At the end of growing season (phase of maturity) negative selection was made in coloured lines and only the plants typical for a given line were picked.

This specialized collection includes over 50 brown cotton lines and over 20 green lines. Brown lines had fiber length of 22.3–26.5 mm and lint percentage 27.2–36.8%, green lines 23.2–27.9 mm and 27.0–34.4%, respectively. Green cotton lines had longer fiber, produced lower seed cotton yield and were later ripening than brown cotton lines.

Specialized collection based on naked seed forms of cotton

Creation of naked seed varieties is of importance for excluding an expensive and environmentally polluting operation of cotton seed preparation technology for sowing – separation of fuzz from seed with sulfuric acid, and this means that such varieties will have great economic and ecological effects.

Sources of naked seed are the interspecific *G. hirsutum* L × *G. barbadense* L. hybridization and the remote hybridiza-

Table 5. Economic indicators of cotton lines with naturally colored green fiber

Line No.	Seed cotton yield (kg ha^{-1})	In % to Chirpan-539	Boll weight (g)	Fiber length (mm)	Lint percentage (%)
Chirpan-539	949	100.0	5.6	26.5	41.5
312	950	100.1	4.9	25.6	30.5
313	650	68.5	5.0	25.3	31.5
314	1348	142.0	4.5	25.6	27.4
316	1053	110.9	4.8	24.6^{00}	30.3
389	644	67.9	4.3^{000}	25.9	30.7
391	547	57.6	5.0	24.3^{00}	31.9
393	751	79.1	5.0	26.1	28.9
394	548	57.7	4.9^{0}	26.0	27.0
395	646	68.1	5.0	24.6^{00}	27.1
562	953	100.4	4.8^{0}	24.5^{00}	29.3
564	946	99.7	4.5^{00}	25.0^{0}	29.6
L.S.D. 5.0%	–	–	0.7	1.4	1.5
L.S.D. 1.0%	–	–	0.9	1.8	2.0
L.S.D. 0.1%	–	–	1.2	2.4	2.6

Source: Authors' own elaboration

tion of *G. hirsutum* L. with the wild species *G. thurberi* Tod. Naked seed forms were selected in the progenies of the allotetraploid *G. thurberi* Tod. × *G. raimondii* Ulbr. Crossing of naked seed forms with brown cotton produced new naked seed forms with naturally brown fiber.

The most distinctive feature of samples in this collection is that they have naked seed without fuzz covering their surface. This quality is very valuable, because in almost all Bulgarian and foreign cotton varieties of the *G. hirsutum* L. species, their seeds have fuzz covering.

In this collection, 6 lines are particularly valuable: No. 81, No. 84, No. 88 and No. 93 with white fiber, No. 105 and No. 106 with brown fiber. Lines No. 81, No. 84 and No. 88 were obtained from the cross of line No. 281 × Sahel-64 (Iranian cultivar). Line No. 93 was selected from line No. 357 originating from line 281 × Giz 1631. Line 281 was obtained from the crossing of the Bulgarian cultivars Beli Izvor × Progress. Line No. 105 and line No. 106 were obtained from the cross of the Bulgarian cultivar Avangard-264 × Eva (Greek). Their brown fiber was the result of natural foreign pollination with brown cotton. The cultivars Progress and Avangard-264 involved in pedigree of the naked seed lines were obtained through interspecific *G. hirsutum* L. × *G. barbadense* hybridization.

Naked seed is a complex conditioned trait and therefore continuous and targeted selection on this trait has been conducted. All naked seed lines belong to the species *G. hirsutum* L. and in earliness they were equal to modern varieties, their vegetation period was 109–125 days, depending on the year conditions, average of 110–115 days.

Lines No. 81, No. 84, No. 88 and No. 93 were characterized by lower productivity, smaller boll weight and lower lint percentage, but had longer fiber compared to modern varieties (Table 6).

Lines No. 105 and No. 106 combine naked seed with brown fiber. These lines are early maturing and mature at the same time with modern varieties. In productivity these lines approached the modern varieties and had smaller boll weight, lower lint percentage and shorter fiber.

Naked-seeded lines, despite showing lower values for some of the investigated characters, are very valuable for selection and cotton production. Lines 105 and 106 can be presented as new candidate-varieties for testing in the IASAS network so far there are no reports in the specialized literature of obtained naked seed brown cotton forms and varieties.

Naked seed cotton forms (without fuze and fiber) were selected in progenies of the allotetraploid *G. thurberi* Tod. × *G. raimondii* Ubrl. (Fig. 1).

Specialized collection based on male-sterile forms for the purposes of heterosis selection in cotton

An important direction in cotton breeding is the use of heterosis to increase productivity. However, the use of heterosis in practice can only be realized with the creation and implementation of heterosis varieties based on male sterility.

Field Crops Institute in Chirpan has nine available male sterile (MS) lines included in this specialized collection. MC

Table 6. Economic qualities of neke-seeded cotton lines in 2015, 2017

Line No.	Seed cotton yield (kg ha^{-1})	In % to Chirpan -539	Boll weight (g)	Fiber length (mm)	Lint percentage (%)
	White cotton				
Chirpan-539	1543	100.0	4.8	25.2	40.5
84	1411	94.1	4.5	26.0	39.6
88	1382	89.6	4.5	26.4	38.2
81	1325	85.9	4.5	25.8	39.2
93	1286	88.3	4.7	25.2	39.5
L.S.D. 5.0%	109	7.1	0.2	0.5	0.5
L.S.D. 1.0%	145	9.4	0.3	0.7	0.7
L.S.D. 0.1%	189	12.2	0.4	0.9	0.9
	Brown cotton				
Chirpan-539	1571	100.0	4.8	25.2	40.5
105	1443	91.9	4.6	23.7	37.9
106	1519	96.7	4.4	23.7	37.1
L.S.D. 5.0%	70	4.5	0.2	0.5	0.5
L.S.D. 1.0%	95	6.0	0.3	0.7	0.7
L.S.D. 0.1%	127	8.1	1.4	0.9	0.9

Source: Authors' own elaboration

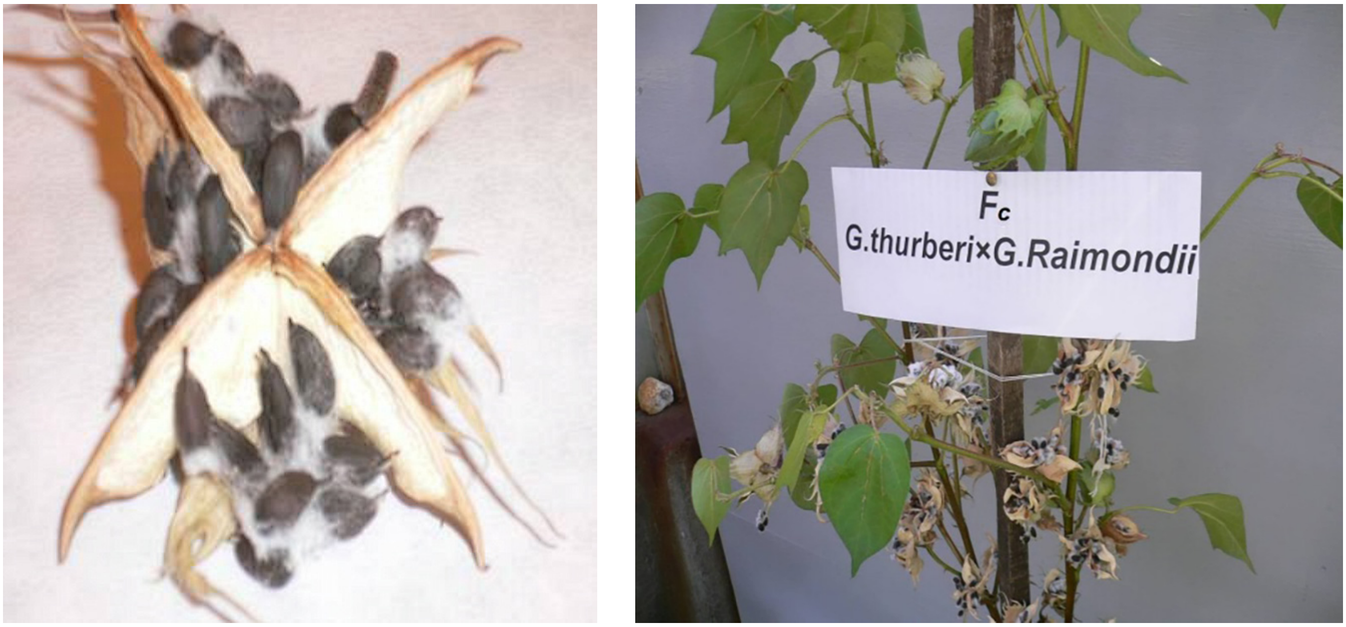

Fig. 1. Naked seed obtained after segregation of the allotetraploid *G. thurberi* Tod. × *G. raimondii* Ubrl.
Source: Authors' own elaboration

lines are characterized by sterile and fertile phase, which allows pollination without castration of flowers and their maintenance through self-pollination. Five MC lines – 107, 108, A-I, A-21 and A-65 were taken from the genetic resources of the Institute in Chirpan. The studies carried out on these lines have shown that MS lines were characterized by difficult bursting of anthers, and pollen was sterile until 2–4 p.m., which can solve the problem with hand emasculation (Stoilova et al., 2006; Stoilova and Rusev, 2007). All sterile lines were characterized by very low productivity, low lint percentage (33.1–35.0%), but in fiber length (28.5–29.9 mm) were superior to modern varieties.

Stoilova et al. (2008) studied the combining ability and found that from the maternal forms – MC lines, MC line A-I emerged as the most suitable for heterosis selection and from the paternal forms these were the cultivar Chirpan-539 and line No. 5 (*G. hirsutum* L.) with positive GCA and high variances of SCA.

Test results of MC lines 273 and 274 (Bulgarian selection) showed that they were very early, with low productivity, low lint percentage (34.0–35.0 %) and long fiber (28.0–29.0 mm).

The collection has been enriched with two new MC Spanish lines FR-H-1003 and FR-H-1004.

Heterosis is extremely important for cotton, especially for countries like ours, with limited temperature and rainfall security. In cotton, heterosis manifestations were observed mainly for productivity and fiber length in some cases also for other trairs.

Some crosses of MS-lines with promising varieties have shown high heterosis effect for both traits (Stoilova, 2008; 2009).

Conclusions

Specialized trait collections based on coloured cotton, naked seed genotypes and male sterility forms have been created to be purposefully used to solve existing serious environmental problems in cotton production and problems in heterosis selection.

Created collections will facilitate the use of available genetic resources relevant to the collections, and are basis for creating new varieties with high economic and ecological effects, as well as high-yielding hybrids based on male-sterility. With these, commercial and other available germplasm will be preserved and the hereditary basis of selection in the relevant directions will be enriched and expanded.

As a result of successful selection, brown cotton varieties Isabell, Egea and Nike as well as some coloured genotypes brown and green had good productivity and good technological fibre properties.

Lines No.105 and No.106 combining two outstanding characteristics naked seed and brown fiber are very valuable for selection and cotton production.

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