

Combining suitable brown lint color, fiber quality, and yield in F₂ cotton hybrids

Volkan Mehmet Çınar^a, Aydın Ünay^{b,*}

^a Postdoctoral Researcher, Faculty of Agriculture, Department of Field Crops, Aydın Adnan Menderes University, Aydın, Türkiye

^b Faculty of Agriculture, Department of Field Crops, Aydın Adnan Menderes University, Aydın, Türkiye

ARTICLE INFO

Keywords:

Brown lint color
Heritability
F₂ hybrids
Gene action
Line × tester

ABSTRACT

Naturally colored cotton is eco-friendly because its use in textiles does not require dyeing, and textiles made from colored cotton are the best for human health. Despite their many advantages, the low yields and unfavorable quality traits of colored cotton varieties limit extensive production. Therefore, F₁ and F₂ populations of inter-specific (*Gossypium hirsutum* L. × *Gossypium barbadense* L.) and intraspecific (*Gossypium hirsutum* L.) cotton hybrids were examined to develop brown-colored cotton varieties with superior traits. Gelincik and Nazilli Deve Tüyü (NDT)-15 varieties (*Gossypium hirsutum* L.) with brown-colored lint were used as female parents. The male parents included Giza-75, Bahar-82 (*Gossypium barbadense* L.), Fiona, May-455, İpek-607 and Claudia (*Gossypium hirsutum* L.) varieties with white-colored lint. According to the line × tester mating design, 12 hybrid combinations were obtained. F₁ populations had higher yield and fiber length values than their parents and F₂ populations. F₂ populations had more unfavorable color values (L: whiteness, a*: red-green, and b*: yellow-blue) than their colored parents but were superior to F₁s. The ratio of general combination ability variance to specific combination ability variance was less than 1, indicating that yield, ginning out-turn, fiber quality, and color parameters were controlled by non-additive genes. In the biplot graph, color and fiber parameters are located in the opposite direction from yield, indicating that it is difficult to develop colored cotton with high yield and fiber quality. Among the 960 F₂ single plants evaluated, ten plants were carefully selected according to the optimization of yield, fiber quality, and color values. Based on potential diallel crosses of the ten selected plants, it was decided to begin a recurrent selection with that population. Furthermore, it was determined that F₂ seeds of Nazilli DT-15 × İpek-607 and Gelincik × Giza-75 combinations may be successfully given to farmers.

1. Introduction

Today, the lint of modern cotton varieties is mainly white, but colored lint is seen in wild cotton breeds and is considered to have evolved to white lint as a result of selection (Fang et al., 2017). Although there are many color options, research has mainly focused on cotton with brown and green-colored lint (Malik et al., 2021; Naoumkina et al., 2024; Wen et al., 2024). Natural-colored fabrics obtained from brown-colored cotton fibers without the need for chemical dyeing are considered to be environment-friendly. These fabrics resist color fading during washing and protect the skin from ultraviolet radiation. Furthermore, textiles made from naturally colored fibers have flame-retardant properties (Hinchliffe et al., 2024). Breeders of cotton with natural-colored fibers are addressing the problem of low yields and poor quality and are constantly working on it. Ecological yarns and

fabrics depend on fiber production within a commercially acceptable range.

Neglect of yield and fiber quality in the cultivation and breeding of cotton with colored lint has led to low yields and adverse quality traits (Katz et al., 1997). The yield of green and brown fiber varieties is 65–80 % of the yield of white fiber cotton varieties (Gong et al., 2018). Although cotton with colored lint has many advantages, the pleiotropic effects of color pigment genes result in thin, short, and weak fibers (Gong et al., 2014; Singh et al., 2001; Tu et al., 2014). Fabrics made from fragile and non-resistant colored cotton lint can be weak, although shorter and finer fibers make them smoother and more comfortable (dos Santos et al., 2023). In classical breeding studies, the negative relationship between color pigmentation and fiber quality traits was tried to be broken by interspecific crosses. In the studies conducted in China, the fiber length of the second-generation colored cotton varieties was

* Corresponding author.

E-mail address: aunay@adu.edu.tr (A. Ünay).

<https://doi.org/10.1016/j.indcrop.2024.119674>

Received 20 June 2024; Received in revised form 16 August 2024; Accepted 14 September 2024

Available online 7 January 2025

0926-6690/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).



Fig. 1. The lint color of Gelincik (left) and Nazilli DT-15 (right) used as female (Çınar et al., 2023).

Table 1
Parental cotton genotypes used in the line × tester mating design.

Lines (brown colored)	Testers (white)
Gelincik (<i>Gossypium hirsutum</i> L.)	Fiona (<i>Gossypium hirsutum</i> L.)
Nazilli DT-15 (<i>Gossypium hirsutum</i> L.)	May-455 (<i>Gossypium hirsutum</i> L.)
	İpek-607 (<i>Gossypium hirsutum</i> L.)
	Claudia (<i>Gossypium hirsutum</i> L.)
	Giza-75 (<i>Gossypium barbadense</i> L.)
	Bahar-82 (<i>Gossypium barbadense</i> L.)

28–29 mm; fiber strength was 21–22.9 cN tex⁻¹; fiber fineness was 4.5 mic., and ginning out-turn 36–40.1 % (Gong et al., 2018). Similarly, although the level of white cotton was reached regarding seed cotton yield, fiber quality traits such as length and strength were insufficient (Jeyaraj et al., 2023; Taufiq Hidayat and Nurindah, 2019). It was emphasized that additive genes were effective for lint color, ginning out-turn, and fiber strength in brown × white hybrids, and single plant selection should be done in early generations (Çınar et al., 2023; Feng et al., 2011). In addition to fiber length, fineness, and strength, lint color should be a key factor in assessing the fiber quality of naturally colored cotton (Zheng et al., 2024).

The brown color was controlled by a single nuclear gene governed by six loci (Lc1 to Lc6) and exhibited dominant inheritance (Carvalho et al., 2014; Patil and Patil, 2021). Lc1 was found to be located on chromosome seven, while Lc2 was found to be linked to chromosome 6. Lc3 was discovered to be a complementary gene that increases the expression of Lc1 and Lc2, giving the mohair a dark brown color (Koehl, 1985).

Classical plant breeding can be ineffective due to inbreeding depression and loss of super alleles. Heterosis has been used as an alternative to homozygous-homogeneous varieties to increase yield and improve quality in many crops. Meredith (1990) emphasized that the difficulty of F₁ seed production, high costs, and low levels of heterosis limit the use of hybrid varieties in cotton (*Gossypium hirsutum* L.). Many studies have suggested the potential use of F₂ hybrids to enhance seed cotton yield via a comparative evaluation of hybrid generations (Chen et al., 2021, 2022; Iqbal et al., 2003, 2008; Meng et al., 2019; Olvery, 1986). It was also found that F₂ hybrids had more adaptation ability than F₁ hybrids and pure lines (Iqbal et al., 2003). In breeding colored lint cotton, second-generation hybrids can be an alternative to the negative link between yield, quality, and color parameters in classical breeding studies. This option has not been considered in any study where colored lint is the primary objective.

The L*, a*, and b* values determined by Chromameter provide more standardized data for the measurement of colored lint than the Rd and +b system and help in color selection and breeding. This study aims to determine the suitable parents and promising hybrids in terms of color values, fiber quality traits, and yield. It is planned that the genetic-statistical estimates obtained as a result of line × tester analysis will guide the evaluation of the population and the breeding method.

2. Material and methods

Two brown-colored varieties used as female parents (Fig. 1) were crossed with six white cotton varieties used as male parents in a line × tester mating design, and 12 F₁ and 12 F₂ were generated in 2021 and 2022, respectively (Table 1).

In 2023, 32 genotypes, including parents, F₁, and F₂ hybrid combinations, were sowed in the Randomized Complete Block Design (RCBD) with four replications. Each plot consisted of six rows, each with a length of 5 m. The sowing norm was 0.70 × 0.10 m. The total trial area was 2688 m².

Cultural practices recommended for cotton cultivation in the Aegean region, such as irrigation, fertilization, and pest control, were performed. From each plot, 50 plants for F₂ hybrid combinations and 20 plants for parents and F₁ hybrids were randomly sampled. In total, 4000 plants were harvested individually, first to determine seed cotton yield per plant (SCY; g) and then ginned with a laboratory-type roller gin to determine ginning out-turn (GOT; %). Lint color and quality traits vary greatly depending on the position of the boll on the plant. For this reason, all bolls of each plant randomly sampled from each plot were collected and put in individual paper bags during the harvest time. L* (whiteness), a* (red-green), and b* (yellow-blue) color values of lint, according to the color scale of The International Commission on Illumination (CIE), were determined using Chromameter CR-400 (Konica Minolta Inc., Japan). Fiber quality traits such as fiber fineness (FF; mic.), fiber length (FL; mm), and fiber strength (FS; g tex⁻¹) were analyzed using USTER High Volume Instruments (HVI-1000, Switzerland).

Analysis of variance (ANOVA) was used to assess the difference among genotypes according to RCBD for the studied traits by the 'agricolae' package developed by de Mendiburu and de Mendiburu (2019) in R studio according to Kempthorne (1957). Orthogonal contrasts were used to compare the grand means of parents, F₁s, and F₂s (JMP® 14., 2018). The data obtained from the studied traits were analyzed separately in F₁ and F₂ generations based on the Line × Tester genetic statistics method (Singh and Chaudhary, 1979), and the variances of combining ability used to determine the gene actions controlling the traits were estimated in TARPOGEN software developed by Özcan and Acikgoz (1999). PCA (Principal component analysis) was performed by the 'FactoMiner' developed by Lê et al. (2008) package in R software.

3. Results and discussion

The present study evaluated the differences among populations in yield, ginning out-turn, lint color, and fiber parameters by line × tester mating design to estimate gene action in the control of traits and identify suitable parents and promising hybrid combinations. The results of line × tester analysis using data recorded from individual plant observations for yield, lint color, and quality characters of parents, F₁ and F₂

Table 2Analysis of variance on parents (P), F₁, and F₂ populations for all traits studied.

SOV	df	Yield and Fiber Quality Traits					Color Parameters		
		SCY	GOT	FL	FF	FS	L*	a*	b*
Blocks	3	1005.5	23.2*	2.5	0.2	3.3	14.2*	1.0	5.4*
Genotypes	31	4328.2**	64.9**	40.8**	0.9**	74.9**	366.2**	37.0**	133.9**
Error	93	521.7	7.2	1.8	0.1	3.3	4.7	0.6	1.8
P vs F ₁	1	1178.9	8.3	33.6**	2.0**	24.3**	1148.4**	116.0**	348.4**
P vs F ₂	1	6302.7**	24.8	49.0**	3.9**	230.5**	325.7**	31.8**	113.5**
F ₁ vs F ₂	1	2537.3*	5.5	204.6**	0.4*	131.3**	878.2**	91.9**	224.8**

^a * $p \leq 0.05$; ** $p \leq 0.01$.^b SCY: Seed cotton yield per plant (g); GOT: Ginning out-turn (%); FL: Fiber length (mm); FF: Fiber fineness (mic.); FS: Fiber strength (g tex⁻¹); L*: Whiteness; a*: Red-green; b*: Yellow-blue.**Table 3**

Mean squares for all traits studied at line × tester crosses.

			Yield and Fiber Quality Traits					Color Parameters		
SOV	df		SCY	FL	FF	FS	GOT	L*	a*	b*
Blocks	3	F ₁	883.0	0.3	0.17*	2.2	7.2	5.8	4.2	1.2
		F ₂	1363.0	3.0	0.24	5.1	22.7	9.5*	4.6	2.6
Crosses	11	F ₁	2735.1**	36.3**	0.90**	27.3**	61.8**	272.1**	23.6**	90.6**
		F ₂	7283.3**	26.0**	0.96**	67.8**	77.9**	231.8**	29.3**	86.4**
Lines	1	F ₁	577.4	35.6**	0.19	98.4**	18.6	2730.9**	223.1**	905.9**
		F ₂	32.6	19.4	1.28	40.5	26.5	551.2	28.7	161.9
Testers	5	F ₁	3620.9	71.6**	1.90**	37.9**	125.9**	35.8	3.9	12.9
		F ₂	8595.0	44.8*	1.01	118.4*	107.9	187.3	40.3	92.9
Line × Testers	5	F ₁	2280.7**	1.0	0.04	2.5	6.2	16.7**	3.3	5.4*
		F ₂	7421.6**	8.6**	0.84**	22.6**	58.2**	212.5**	18.6**	64.6**
Error	57	F ₁	409.1	1.00	0.04	2.2	3.7	4.8	4.8	1.9
		F ₂	622.5	2.3	0.10	3.8	9.4	3.3	4.5	1.3
$\sigma^2_{\text{GCA}} / \sigma^2_{\text{SCA}}$		F ₁	0.06	112.00	−49.00	15.59	5.07	4.90	−3.20	5.51
		F ₂	−0.01	0.64	0.38	0.55	0.09	0.1	0.28	0.08
Cont. of lines (%)		F ₁	1.92	8.93	1.90	32.76	2.74	91.2	85.96	90.79
		F ₂	0.04	6.77	12.17	5.43	3.10	21.6	8.79	17.07
Cont. of testers (%)		F ₁	60.18	89.77	96.06	63.02	92.65	5.9	7.69	6.49
		F ₂	53.64	78.28	47.95	79.43	62.93	36.7	62.47	49.01
Cont. of L × T (%)		F ₁	37.90	1.30	2.04	4.23	4.61	2.8	6.36	2.73
		F ₂	46.32	14.95	39.88	15.14	33.97	41.7	28.74	33.92

^a * $p \leq 0.05$; ** $p \leq 0.01$.^b GCA: General combining ability; SCA: Specific combining ability; SCY: Seed cotton yield per plant (g); GOT: Ginning out-turn (%); FL: Fiber length (mm); FF: Fiber fineness (mic.); FS: Fiber strength (g tex⁻¹); L*: Whiteness; a*: Red-green; b*: Yellow-blue.

populations, and population comparisons are presented in Table 2.

Significant genotypic differences for all traits indicate a wide variation. Although the ginning out-turns of all genotypes were similar, seed cotton yield and fiber length of F₁ were significantly superior compared with their parents and F₂ (Table 4). F₂ hybrid combinations were found to have lower fiber length and fiber strength. Regarding color parameters, it was observed that F₂ approached the parent averages compared with F₁ (Table 4).

ANOVA for combining ability (Table 3) indicated that the differences between hybrids for all studied traits were significant. Differences due to lines were significant in the F₁ generation but not in the F₂ generation. The difference between both generations indicates different gene effects in trait control. In the segregating F₂ population, more line × tester interaction effect is observed than line and tester parents due to non-additive gene effects. In addition, the difference between testers was significant only for FL and FS in the F₂ generation. The most meaningful result was that the line × tester effect in F₂ generation was significant for all traits examined, and the contribution of the line × tester to the variance was high. The studied traits were clearly under the action of non-additive genes in the F₂ generation. The results of this study are in agreement with Tausif (2008), Giri et al. (2020), Ganvit et al. (2024), Vanapariya et al. (2022), El-Shazly et al. (2024), Baghyalakshmi et al. (2024) who found non-additive gene effects significant for seed cotton yield and ginning out-turn.

All color parameters (L, a*, and b*) are controlled by non-additive

genes in the F₂ generation in contrast to F₁, indicating increased segregation variance in the F₂ generation. According to the line × tester mating design, homozygous-homogeneous and heterozygous-homogeneous populations were studied in F₁, while heterozygous-heterogeneous populations were also included in F₂ (Lande, 1981). In our previous study, additive gene effects were predominant for SCY, GOT, fiber quality traits, and color parameters in F₁ generation (Çınar et al., 2023). In the F₂ generation, unlike the F₁ generation, epistasis and dominance in the F₁ generation may be responsible for this, and the possibility of recessive alleles may support this (Falconer and Mackay, 1996; Klug et al., 2018). It was stated that the additive, dominant model is valid in the inheritance of all traits, including lint color, and that dominant effects are higher. Therefore, it was emphasized that direct selection would not be effective in breeding these traits and that recurrent selection would be useful for developing colored cotton hybrids (Baghyalakshmi et al., 2024; Khan et al., 2009). In parallel with the recommendations of the authors mentioned above, this study was planned to determine the most superior single plants to form the starting material for recurrent selection due to non-additive effects.

The SCY data in Table 3 show that barbadense parents have the lowest yields, whereas hirsutum and cultivated parents have high yields, and colored parents are between both groups. Colored × barbadense hybrids produced low yields in both generations, indicating that the effect of barbadense parents on yield increase was limited. In the F₁ generation, Gelincik × Fiona, Gelincik × May-455, Gelincik × İpek-607,

Table 4

Genotype mean performance for all traits studied.

Genotype	Yield and Fiber Quality Traits					Color Parameters		
	SCY	GOT	FL	FF	FS	L*	a*	b*
Parents								
Gelincik	99.95	42.14	24.65	5.20	22.01	59.60	9.70	26.38
NDT-15	98.87	38.16	26.15	5.04	24.11	70.29	5.84	21.48
Giza-75	62.60	33.87	35.24	4.50	39.86	86.36	0.89	11.23
Bahar-82	80.94	39.01	34.61	4.33	37.85	85.02	1.36	13.02
Fiona	124.35	44.28	29.64	4.96	32.08	89.40	0.21	8.71
May-455	119.82	44.46	29.12	4.99	30.78	88.32	0.34	8.85
İpek-607	131.74	37.21	30.30	5.04	31.02	90.18	0.21	7.76
Claudia	129.63	44.64	29.40	5.04	31.75	90.00	0.31	8.35
F ₁								
Gelincik × Giza-75	69.73	36.54	34.44	3.89	31.02	71.21	4.49	20.03
Gelincik × Bahar-82	55.03	36.97	33.28	4.32	31.68	67.69	5.08	20.98
Gelincik × Fiona	114.01	44.80	28.07	5.20	27.34	70.79	5.92	20.77
Gelincik × May-455	114.05	41.88	27.64	4.90	25.94	67.61	7.75	23.28
Gelincik × İpek-607	101.37	39.86	28.94	4.81	27.15	68.26	6.95	23.23
Gelincik × Claudia	95.54	42.57	29.73	4.65	28.61	76.48	4.70	18.59
NDT-15 × Giza-75	60.07	31.98	36.31	3.67	34.89	86.00	1.24	11.51
NDT-15 × Bahar-82	51.91	35.18	35.88	4.06	33.59	83.38	2.05	14.13
NDT-15 × Fiona	114.08	44.86	30.40	5.02	30.52	86.86	1.24	11.68
NDT-15 × May-455	101.99	41.13	28.88	4.79	29.65	85.88	1.77	13.55
NDT-15 × İpek-607	143.40	40.06	30.61	4.70	30.61	84.38	1.37	12.18
NDT-15 × Claudia	96.65	41.92	30.36	4.79	29.67	86.07	1.36	11.73
F ₂								
Gelincik × Giza-75	53.75	38.55	30.24	4.68	31.94	69.69	4.96	20.27
Gelincik × Bahar-82	30.34	38.76	29.15	4.39	28.72	66.82	5.03	20.87
Gelincik × Fiona	118.67	45.40	27.42	5.26	27.48	71.16	5.85	20.65
Gelincik × May-455	95.40	38.84	25.10	4.20	22.13	65.51	8.12	23.50
Gelincik × İpek-607	100.60	38.68	26.87	4.79	25.12	64.44	8.53	24.34
Gelincik × Claudia	123.52	40.23	27.17	4.30	25.42	73.06	5.83	20.59
NDT-15 × Giza-75	21.87	29.24	34.15	3.74	35.42	76.28	2.52	15.37
NDT-15 × Bahar-82	56.71	45.83	29.16	5.08	31.31	88.90	0.37	8.57
NDT-15 × Fiona	147.62	43.55	25.70	4.22	23.02	80.95	9.34	24.82
NDT-15 × May-455	112.39	38.45	25.60	4.00	23.09	63.70	8.64	24.12
NDT-15 × İpek-607	104.68	38.56	28.84	4.83	28.44	65.83	4.55	18.45
NDT-15 × Claudia	28.90	35.91	30.11	3.80	30.52	75.68	3.66	16.84
Tukey HSD _(0.05)	63.05	7.38	3.74	0.78	5.03	5.99	2.11	3.70
x ⁻ (parents)	105.99	40.47	29.89	4.89	31.18	64.94	7.77	23.93
x ⁻ F ₁ s	98.15	39.81	31.21	4.57	30.06	77.88	3.66	16.81
x ⁻ F ₂ s	87.87	39.33	28.29	4.44	27.72	71.83	5.62	19.87
x ⁻ (grand)	96.26	39.80	29.79	4.60	29.46	76.74	4.07	17.06

^a SCY: Seed cotton yield per plant (g); GOT: Ginning out-turn (%); FL: Fiber length (mm); FF: Fiber fineness (mic.); FS: Fiber strength (g tex⁻¹); L*: Whiteness; a*: Red-green; b*: Yellow-blue.

Nazilli DT-15 × Fiona, Nazilli DT-15 × May-455, and Nazilli DT-15 × İpek-607 produced the highest yields. In F₂, these were added to the Gelincik × Claudia hybrid. For a yielding-colored genotype, hirsutum hybrids appeared to be essential.

Except for barbadense hybrids, the ginning out-turn of all F₁ combinations was above 40 %. Gelincik × Fiona, Nazilli DT-15 × Fiona, Gelincik × Claudia, and Nazilli DT-15 × Bahar-82 F₂ combinations also exhibited ginning out-turn above 40 %. In contrast, to yield and ginning out-turn, as expected, the most superior parents in terms of length, fineness, and strength were the parents belonging to barbadense species, and colored × barbadense hybrids showed superior performance both in F₁ and F₂. In F₂, Nazilli DT-15 × Claudia hybrid was added to these hybrids. Hybridization between different species has been proposed to generate higher lint color variation and obtain superior genotypes (Revanasiddayya et al., 2024). Shi et al. (2016) and Lu et al. (2017) emphasized that *Gossypium barbadense*, which is evaluated for its superior fiber quality traits, has low lint yield and limited adaptability. Nie et al. (2020) reported the results of numerous studies with hirsutum × barbadense hybrids and indicated that a yield-enhancing locus in *Gossypium barbadense* varieties may be derived from *Gossypium hirsutum*. Wang et al. (2022) reported that barbadense type cottons were grouped into four groups, namely US/Pima, Egyptian, Central Asian, and Modern barbadense varieties and that modern varieties have superior fiber properties than their predecessors. It was concluded that increasing the

number of varieties in hybridization studies with barbadense would be useful.

The most important color parameters in the chromameter are L* (whiteness), a* (red-green), and b* (yellow-blue) color values of the lint. In the breeding of colored lint, low L* values are desired, but high a* and b* values are desired. Gelincik had better color performance than Nazilli DT-15. F₁ combinations with the Gelincik as the parent had superior values for all color parameters. Along with the Gelincik combinations, F₂ combinations such as Nazilli DT-15 × May-455 and Nazilli DT-15 × İpek-607 were also genotypes with superior color performance. However, it should be noted that none of the combinations reached the perfect color value of the Gelincik. Evaluation in terms of all three-color criteria showed that Nazilli DT-15 × May-455, Gelincik × İpek-607, and Gelincik × May-455 hybrid combinations had superior traits. Joshi and Chirde (1998) stated that the most important goal in colored lint cotton breeding is to obtain superior hybrids from colored parents, but this is prevented by the negative relationship caused by pleiotropy, and as a result, sufficient heterosis does not occur. The yield, ginning out-turn, and fiber quality traits of colored lint cotton obtained as a result of the breeding studies carried out in recent years are important in terms of showing the level reached. Wen et al. (2024) reported that 67 natural-colored cotton varieties had been developed since 2000, and brown cotton varieties have reached 80 % yield level of white cotton varieties. GOT and FL of dark brown Chinese varieties were reported as



Fig. 2. PCA values in the biplot diagram for all genotypes and traits studied in the F₁.

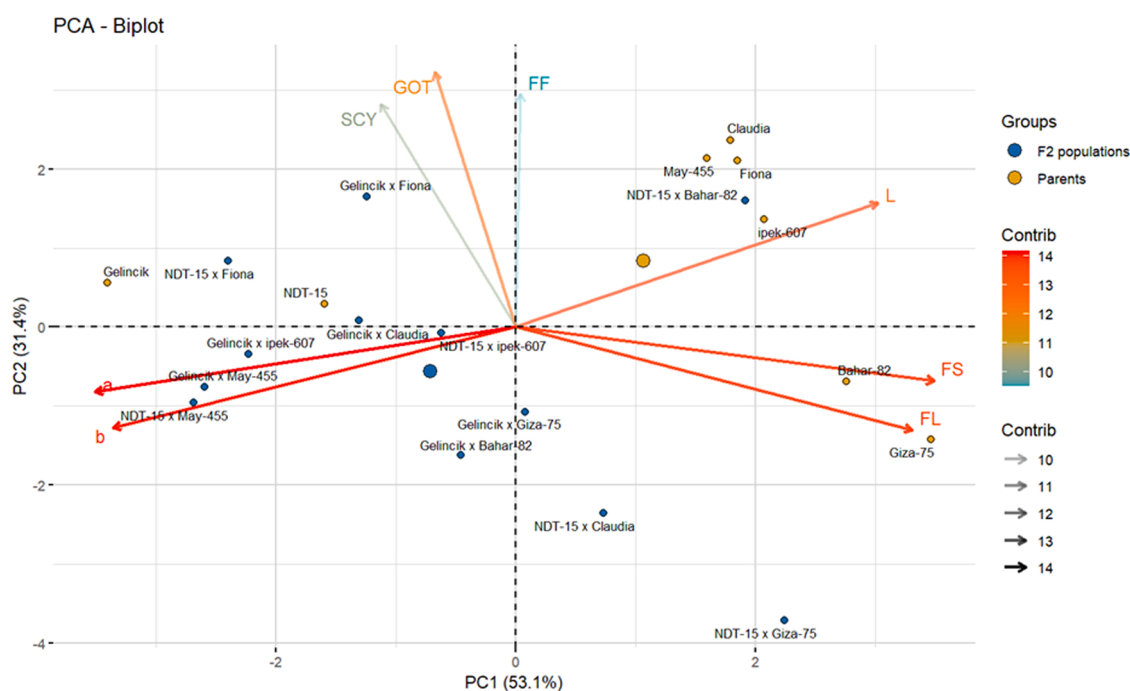


Fig. 3. PCA values in the biplot diagram for all genotypes and traits studied in the F₂.

41.7 % and 30.8 mm (Sun et al., 2021), while the traits of Uzbek varieties were 36.1 % and 28.4 mm, respectively (Rahimova et al., 2024). Gelincik × Giza-75 and Gelincik × Bahar-82 are super light brown lint genotypes with superior ginning out-turn and fiber quality than Chinese and Uzbek varieties except for yield.

Principal component analysis (PCA) has been used by numerous researchers in cotton (Aslam et al., 2017; Graffelman and Aluja-Banet, 2003; Malik et al., 2013; Munir et al., 2020; Rathinavel, 2018) as an efficient method among biometric procedures to assign each genotype to only one group and at the same time reflect the importance of the largest

contributor to the total variability in each axis of differentiation. PCA, which is used in breeding studies, provides important results in determining which traits the improved genotypes are combined. It also reveals the direction of the relationship between traits such as colored lint, fiber quality traits, and yield, as in this study. A biplot diagram of the principal components analysis of F₁ and F₂ populations according to the yield, lint color, and fiber quality parameters is given in Figs. 2 and 3. In F₁ populations, the largest variance (54.1 %) was recorded in the first principal component (PC1). PC1 and PC2 explained 91.3 % of the total variation for F₁ populations, whereas the PC1 and PC2 axes described

Table 5Traits of selected F₂ single plants.

Genotype	SCY	GOT	FL	FF	FS	L*	a*	b*
Gelincik × İpek-607 ⁽²⁾	71.30	37.45	29.42	4.78	30.79	57.75	12.40	31.12
Gelincik × Bahar-82 ⁽²⁾	50.20	38.45	27.14	3.19	29.02	60.54	4.80	21.84
Gelincik × Claudia ⁽²⁾	47.90	42.38	27.35	3.90	32.59	65.61	6.68	22.76
Gelincik × May-455 ⁽²⁾	111.50	41.70	27.46	4.50	29.56	69.67	6.65	23.73
Gelincik × Bahar-82 ⁽¹⁾	70.40	47.87	30.09	3.54	28.92	67.23	6.18	20.95
NDT-15 × İpek-607 ⁽²⁾	76.60	37.08	30.86	4.33	29.07	67.10	6.64	22.71
NDT-15 × İpek-607 ⁽³⁾	140.00	42.86	27.77	4.47	31.22	66.80	6.96	24.79
NDT-15 × İpek-607 ⁽⁴⁾	128.00	42.97	29.22	5.06	28.58	69.55	6.54	22.07
NDT-15 × İpek-607 ⁽⁶⁾	56.00	38.75	28.53	4.52	30.65	67.18	6.06	23.03
NDT-15 × May-455 ⁽¹⁾	78.70	42.44	30.44	3.80	30.57	66.04	8.02	24.85

^a SCY: Seed cotton yield per plant (g); GOT: Ginning out-turn (%); FL: Fiber length (mm); FF: Fiber fineness (mic.); FS: Fiber strength (g tex⁻¹); L*: Whiteness; a*: Red-green; b*: Yellow-blue.

^b The numbers in parentheses next to the genotype names indicate their order in the total eighty plants observed.

84.5 % of the total variation in F₂ populations.

Vector lengths of lint color parameters and fiber quality traits indicated a wider variation than SCY and GOT in F₁ and F₂. In both generations, SCY, GOT, and FF were in the narrow-angle group, whereas color parameters such as a* and b* and FS and FL were in the wide-angle positions.

Biplot plots indicated that the Gelincik was more prominent in directions a* and b* with Nazilli DT-15. The *Gossypium barbadense* varieties, Giza-75 and Bahar-82, clearly synchronized with FS and FL. *Gossypium hirsutum* varieties Fiona, Claudia, İpek-607, and May-455 were located between the blocks formed by SCY, GOT, FF, and L*. In the F₁ generation, Gelincik × Claudia, Gelincik × İpek-607, and Gelincik × May-455 were close to the blocks of a* and b*. In the F₂ generation, Nazilli DT-15 × May-455 and Nazilli DT-15 × İpek-607 hybrid combinations were added to these genotypes. Gelincik × Giza-75, Gelincik × Bahar-82, Nazilli DT-15 × Claudia, and Nazilli DT-15 × İpek-607 F₂ hybrid combinations were found to be located in the middle and close to all traits.

The study evaluated a total of 960 F₂ single plants, 80 plants from each combination. Among these, ten plants with optimum values for all traits were selected (Table 5).

Single plant yield varied between 47.90 g and 140.00 g; ginning out-turn between 37.08 % and 42.97 %; fiber length between 27.14 mm and 30.86 mm; fiber fineness between 3.19 mic. and 5.06 mic.; fiber strength between 28.58 g tex⁻¹ and 32.59 g tex⁻¹. The color measurement parameters L* value ranged between 57.75 and 69.67, a* value between 4.80 and 12.40, and b* value between 20.95 and 31.12. The selection intensity applied in this generation was realized as 1.05 %. Among the varieties used as males in the selected hybrid combinations, İpek-607 attracted attention.

The appearance of different hybrid combinations for each trait and the influence of non-additive genes in the inheritance of traits may limit variability and lead to loss of dominance in early generations. Therefore, the top ten super F₂ single plants were identified to initiate the first cycle of recurrent selection by performing half-diallel crosses to obtain 45 hybrid combinations among selfed populations in F₃, as practiced by Abou El-Yazied et al. (2014) and Balci et al. (2021). In the study by Janbhale et al. (1998), the selection was made for different shades of brown color and fiber quality traits, and recurrent selection was proposed. Similarly, it has been shown that recurrent selection may be a suitable breeding strategy to further improve varieties with colored lint (Malik et al., 2013).

Unstable results on male sterility, fertility restoration, male gametocytes (Chen et al., 2022; Iqbal et al., 2003), and high production cost in conventional hybridization (Singh et al., 2022) prevent the utilization of heterosis breeding in cotton. F₂ combinations that maintain dominance and have the least difference in color and quality between plants can be considered second-generation hybrids. As a result of this study, Gelincik × Giza-75 and Nazilli DT-15 × İpek-607 F₂ combinations were detected

as second-generation hybrids. Although Nazilli DT-15 × İpek-607 F₂ hybrids had higher yield than both colored parents, the ginning out-turn was between the two parents. The fiber of this hybrid was about 2 – 4 mm longer and 4 – 6 g tex⁻¹ more strength than both parents. Gelincik × Giza-75 is low-yielding but has longer and stronger fibers than both colored parents and the Nazilli DT-15 × İpek-607 combination. Meredith (1990), Tang et al. (1993), and Chen et al. (2022) demonstrated that high seed cotton yield and superior fiber quality F₂ hybrids can be successfully used in cotton farming. Several studies have demonstrated that F₂ hybrids retain some of their competitive advantages over their parents (Chen et al., 2021; Meng et al., 2019). The heterogeneity of Gelincik × Giza-75 and Nazilli DT-15 × İpek-607 F₂ combinations has the potential to increase their adaptability and reduce hybrid production costs.

4. Conclusions

The present study revealed that dark brown color, fiber quality traits within commercial limits, high yield, and ginning out-turn are unlikely to be combined in one genotype. The predominance of non-additive genes in the inheritance of mentioned traits and the risk of losing superior, dominant types by self-selection showed that recurrent selection could be successful. It was concluded that F₂ seeds of hybrids with brown-colored lint with ginning out-turn and fiber quality traits close to cotton with white-colored lint and yielding F₂ seeds could be obtained if one shade of color values is compromised. The number of cotton species and parents used in this study is limited. In further studies, the number of wild species and parents with brown-colored lint genes should be increased to break the negative association between color parameters and yield and fiber quality traits, and the possibility of creating more variation should be tried.

Funding

This study was supported by The Scientific and Technological Research Council of Türkiye (TÜBİTAK), with the project no. 221O225. The authors gratefully thank the TÜBİTAK.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Aydın UNAY reports financial support was provided by Scientific and Technological Research Council of Türkiye. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

References

- Abou El-Yazied, M.A., Soliman, Y.A., El-Mansy, Y.M., 2014. Effectiveness of recurrent selection for improvement of some economic characters in Egyptian cotton. *Egypt. J. Agr. Res.* 92 (1), 135–151. <https://doi.org/10.21608/ejar.2014.154473>.
- Aslam, M., Maqbool, M.A., Zaman, Q.U., Shahid, M., Akhtar, M.A., Rana, A.S., 2017. Comparison of different tolerance indices and PCA biplot analysis for assessment of salinity tolerance in lentil (*Lens culinaris*) genotypes. *Int. J. Agr. Biol.* 19 (3), 470–478. <https://doi.org/10.17957/IJAB/15.0308>.
- Baghyalakshmi, K., Priyanka, A.R., Aravind, K., Manickam, S., 2024. Genetic analysis for estimating combining ability in *Gossypium hirsutum* L. × *Gossypium barbadense* L. hybrids. *Electron. J. Plant Breed.* 15 (1), 21–30. <https://doi.org/10.37992/2024.1501.002>.
- Balci, S., Çınar, V.M., Ünay, A., 2021. The effects of modified recurrent selection on fiber characteristics and neps in cotton (*Gossypium hirsutum* L.). *Anadolu J. AARI* 31 (2), 137–142. <https://doi.org/10.18615/anadolu.1029812>.
- Carvalho, L., Farias, F., Lima, M., Rodrigues, J., 2014. Inheritance of different fiber colors in cotton (*Gossypium barbadense* L.). *Crop Breed. Appl. Biot.* 14 (4), 256–260. <https://doi.org/10.1590/1984-70332014v14n4n40>.
- Chen, L., Tang, H., Zhang, X., Qi, T., Guo, L., Shahzad, K., Wang, H., Qiao, X., Zang, R., Zhang, M., Wu, J., 2022. Comparative performance of hybrid generations reveals the potential application of F₂ hybrids in upland cotton. *J. Cotton Res.* 5 (1), 18. <https://doi.org/10.1186/s42397-022-00125-8>.
- Chen, S., Zhao, L., Di, J.C., 2021. Study on advantages of economic traits in F₁ and F₂ generations of parental dominant low-phenol insect-resistant hybrid cotton. *Jiangsu Agr. Sci.* 49 (16), 97–100. <https://doi.org/10.15889/j.issn.1002-1302.2021.16.017>.
- Çınar, V.M., Balci, S., Şimşek, M.K., Ünay, A., 2023. Breeding of brown cotton for spinnable yarn in intraspecific and interspecific hybrids (*G. hirsutum* L. and *G. barbadense* L.). *Ind. Crops Prod.* 202, 116995. <https://doi.org/10.1016/j.indcrop.2023.116995>.
- de Mendiburu, F., de Mendiburu, M.F. 2019. Package 'agricolae'. R Package, Version, 1, 3. Available from: (<https://cran.r-project.org/web/packages/agricolae/agricolae.pdf>).
- dos Santos, M.G., dos Santos, G.L., Bezerra, L.R.F.C., Júnior, A.P.B., da Silveira, L.M., de Lima Tartaglia, F., Lins, H.A., de Araújo Rangel Lopes, W., Souza, Ê.G.F., da Rocha, A.T., dos Santos Coelho, E., 2023. Yield and quality of naturally colored cotton fiber fertilized with phosphorus rates in the Brazilian semiarid. *J. Nat. Fibers* 20 (2), 2214385. <https://doi.org/10.1080/15440478.2023.2214385>.
- El-Shazly, M.W., Mabrouk, A.H., Zaater, M.M., 2024. Generation mean analysis for some traits in two Egyptian cotton crosses. *J. Agr. Chem. Biotechnol.* 15 (3), 35–39. <https://doi.org/10.21608/jacb.2024.263645.1075>.
- Falconer, D.S., Mackay, T.F.C. 1996. Introduction to Quantitative Genetics. Longman.
- Fang, L., Wang, Q., Hu, Y., Jia, Y., Chen, J., Liu, B., Zhang, Z., Guan, X., Chen, S., Zhou, B., Mei, G., 2017. Genomic analyses in cotton identify signatures of selection and loci associated with fiber quality and yield traits. *Nat. Genet.* 49 (7), 1089–1098. <https://doi.org/10.1038/ng.3887>.
- Feng, H.J., Sun, J.L., Wang, J., Jia, Y.H., Zhang, X.Y., Pang, B.Y., Du, X.M., 2011. Genetic effects and heterosis of the fibre colour and quality of brown cotton (*Gossypium hirsutum* L.). *Plant Breed.* 130 (4), 450–456. <https://doi.org/10.1111/j.1439-0523.2010.01842.x>.
- Ganvit, R.S., Chaudhari, K.N., Panchal, R.J., Patel, H.R., Patel, A., 2024. Combining ability and gene action studies for yield and its component traits over different environments in cotton (*G. hirsutum* L.). *Biol. Forum. Int. J.* 16 (4), 154–165.
- Giri, R.K., Verma, S.K., Yadav, J.P., 2020. Combining ability analysis for yield and its contributing traits based on multi-environment testing in upland cotton (*G. hirsutum* L.). *Electron. J. Plant Breed.* 11 (2), 416–424. <https://doi.org/10.37992/2020.1102.073>.
- Gong, W., Du, X., Jia, Y., Pan, Z., 2018. Color cotton and its utilization in China. In: Fang, D. (Ed.), *Cotton Fiber: Physics, Chemistry and Biology*. Springer, Cham, pp. 117–132. https://doi.org/10.1007/978-3-030-00871-0_6.
- Gong, W., He, S., Tian, J., Sun, J., Pan, Z., Jia, Y., Sun, G., Du, X., 2014. Comparison of the transcriptome between two cotton lines of different fiber color and quality. *PLoS One* 9 (11), e112966. <https://doi.org/10.1371/journal.pone.0112966>.
- Graffelman, J., Aluja-Banet, T., 2003. Optimal representation of supplementary variables in biplots from principal component analysis and correspondence analysis. *Biom. J. J. Math. Methods Biosci.* 45 (4), 491–509. <https://doi.org/10.1002/bimj.200390027>.
- Hinchliffe, D.J., Naoumkina, M., Thyssen, G.N., Nam, S., Chang, S., McCarty, J.C., Jenkins, J.N., 2024. Multi-omics analysis of pigmentation related to proanthocyanidin biosynthesis in brown cotton (*Gossypium hirsutum* L.). *Front. Plant Sci.* 15, 1372232. <https://doi.org/10.3389/fpls.2024.1372232>.
- Iqbal, M., Hayat, K., Atiq, M., Khan, N.I., 2008. Evaluation and prospects of F₂ genotypes of cotton (*Gossypium hirsutum* L.) for yield and yield components. *Int. J. Agr. Biol.* 10 (4), 442–446.
- Iqbal, M., Iqbal, M.Z., Chang, M.A., Hayat, K., 2003. Yield and fiber quality potential for second generation cotton hybrids. *Pak. J. Biol. Sci.* 6 (22), 1883–1887.
- Janbhale, N.D., Paralkar, R.M., Yadav, N.V., Darade, R.S., Yadav, M.D., Mehrete, S.S., 1998. Genetics and improvement of lint characters in naturally coloured cotton (*Gossypium hirsutum* L.). *Proc. of the World Cotton Res. Conference-2* (pp. 233–234). Athens, Greece, p. 1998. September 6–12.
- Jeyaraj, R.P., Anantharaju, P., Subramanian, A., Somasundaram, S., Chitra, N., Premalatha, N., 2023. Evaluation on genetic variability and trait association in naturally coloured cotton (*Gossypium hirsutum* L.). *Electron. J. Plant Breed.* 14 (3), 850–856. <https://doi.org/10.37992/2023.1403.095>.
- JMP® 14. 2018. SAS Institute Inc. 2018. JMP Statistical Software, Version 14. Cary, USA.
- Joshi, K.A., Chirde, S.S. 1998. Colour cotton research in Mahyco, India. In *Proc. of the World Cotton Res. Conference-2* (pp. 186–187). Athens, Greece, September 6–12.
- Katz, D., Boone, N., Vreeland, J.J., 1997. Organically grown and naturally colored cotton: a global overview. In: *Proc. of the Beltwide Cotton Conference, Vol. 1*. National Cotton Council, Memphis, TN, pp. 293–297.
- Kempthorne, O., 1957. An introduction to genetics statistics. The Iowa State University Press. John Wiley and Sons, Inc, New York, pp. 453–471.
- Khan, A.A., Azhar, F.M., Khan, I.A., Riaz, A.H., Athar, M., 2009. Genetic basis of variation for lint color, yield, and quality in cotton (*Gossypium hirsutum* L.). *Plant Biosys. – Int. J. Deal. All Asp. Plant Biol.* 143 (supl), 17–24. <https://doi.org/10.1080/11263500903178158>.
- Klug, W.S., Cummings, M.R., Spencer, C.A., Palladino, M.A. 2018. Concepts of Genetics. Pearson.
- Kohel, R., 1985. Genetic analysis of fiber color variants in cotton. *Crop Sci.* 25, 793–797. <https://doi.org/10.2135/cropsci1985.0011183X0025000500017x>.
- Lande, R., 1981. The minimum number of genes contributing to quantitative variation between and within populations. *Genetics* 99, 541–553.
- Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. *J. Stat. Softw.* 25 (1), 1–18. <https://doi.org/10.18637/jss.v025.i01>.
- Lu, Q., Shi, Y., Xiao, X., Li, P., Gong, W., Liu, A., Shang, H., Li, J., Ge, Q., Song, W., 2017. Transcriptome analysis suggests that chromosome introgression fragments from Sea Island cotton (*Gossypium barbadense*) increase fiber strength in Upland cotton (*Gossypium hirsutum*). *G3-Genes Genom. Genet.* 7, 3469–3479. <https://doi.org/10.1534/g3.117.300108>.
- Malik, W., Anjum, N., Usman Khan, M., Abid, M.A., Ashraf, J., Zhang, R., Liang, C., Hanif, M., Qayyum, A., Rahman, M.U., 2021. Genomics of naturally colored cotton: A way forward to initiate precision breeding. In *Cotton Precision Breeding*. Springer International Publishing, Cham, pp. 173–195. https://doi.org/10.1007/978-3-030-64504-5_8.
- Malik, W., Khan, A.A., Sadia, B., 2013. In situ characterization of coloured cotton genotypes. *Aust. J. Crop Sci.* 7 (3), 299–304.
- Meng, Q.Z., Yi, X.D., Zhang, T., et al., 2019. Application prospect of hybrid cotton F₂ in cotton production in Xinjiang. *Anhui Agr. Sci. Bull.* 25 (21), 54–58. <https://doi.org/10.16377/j.cnki.issn1007-7731.2019.21.018>.
- Meredith Jr, W.R., 1990. Yield and fiber-quality potential for second-generation cotton hybrids. *Crop Sci.* 30 (5), 1045–1048. <https://doi.org/10.2135/cropsci1990.0011183X0030000500018x>.
- Munir, S., Qureshi, M.K., Shahzad, A.N., Nawaz, I., Anjam, S., Rasul, S., Zulfikar, M.A., 2020. Genetic dissection of interspecific and intraspecific hybrids of cotton for morpho-yield and fiber traits using multivariate analysis. *Pak. J. Agr. Res.* 33 (1), 9–16. <https://doi.org/10.17582/journal.pjar/2020/33.1.9.16>.
- Naoumkina, M., Hinchliffe, D.J., Thyssen, G.N., 2024. Naturally colored cotton for wearable applications. *Front. Plant Sci.* 15, 1350405. <https://doi.org/10.3389/fpls.2024.1350405>.
- Nie, X., Wen, T., Shao, P., Tang, B., Nuriman-guli, A., Yu, Y., Du, X., You, C., Lin, Z., 2020. High-density genetic variation maps reveal the correlation between asymmetric interspecific introgressions and improvement of agronomic traits in Upland and Pima cotton varieties developed in Xinjiang, China. *Plant J.* 103, 677–689. <https://doi.org/10.1111/tpj.14760>.
- Olvery, J.M., 1986. Performance and potential of F₂ hybrids. In: Nelson, T.C. (Ed.), *Proc. of the Beltwide Cotton Res. Conference*. Las Vegas, NV, January 4–9, 1986. National Cotton Council of America, Memphis, TN, pp. 101–102.
- Ozcan, K., Acikgoz, N., 1999. A statistical analysis program for population genetics. *Proc. of the Symp. Comput. Appl. for Agr. Kucurova University, Adana, Turkey*, pp. 3–6.
- Patil, K.A., Patil, R.S., 2021. The inheritance pattern of brown lint in color-cotton. *Int. J. Curr. Microbiol. Appl. Sci.* 10 (1), 946–950. <https://doi.org/10.20546/ijcmas.2021.1001.114>.
- Rahimova, G., Nabiev, S., Azimov, A., Sagdiev, M., Khamdullaev, S., Shavkiev, J., 2024. Morphological and agronomic characterization of colored cotton cultivars of *Gossypium hirsutum* L. *J. Wildl. Biot.* 8 (2), 165–178. <https://doi.org/10.5281/zenodo.11098144>.
- Rathinavel, K., 2018. Principal component analysis with quantitative traits in extant cotton varieties (*Gossypium hirsutum* L.) and parental lines for diversity. *Curr. Agr. Res.* 3 (6), 54. <https://doi.org/10.12944/CARJ.6.1.07>.
- Revanasiddappa, J.M.N., Fakrudin, B., Kuchanur, P., Yogeesh, L.N., Hanchinal, S., Suma, T.C., Sunkad, G., Muralidhara, B., Maheshkumar, D., Channabasava, S.P., Rashmi, S., 2024. Genetic diversity among coloured cotton genotypes in relation to their fibre colour and ploidy level based on SSR markers. *Czech J. Genet. Plant Breed.* 60, 12–24. <https://doi.org/10.17221/12/2023-CJGPB>.
- Shi, Y., Zhang, B., Liu, A., Li, W., Li, J., Lu, Q., Zhang, Z., Li, S., Gong, W., Shang, H., Gong, J., 2016. Quantitative trait loci analysis of Verticillium wilt resistance in interspecific backcross populations of *Gossypium hirsutum* × *Gossypium barbadense*. *BMC Genom.* 17, 877. <https://doi.org/10.1186/s12864-016-3128-x>.
- Singh, G., Bajwan, A., Sain, S., 2022. An economic analysis of production and marketing of cotton in Haryana. *Pharma Int. J.* 11 (12), 6329–6335.
- Singh, B.D., Chaudhary, R.K., 1979. Line × tester analysis. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi, pp. 205–214.
- Singh, P., Singh, V.V., Waghmare, V.N. 2001. Naturally coloured cotton. *CICR Tec. Bull.* No: 4.
- Sun, J., Sun, Y., Zhu, Q.H., 2021. Breeding next-generation naturally colored cotton. *Trends Plant Sci.* 26 (6), 539–542. <https://doi.org/10.1016/j.tplants.2021.03.007>.

- Tang, B., Jenkins, J.N., McCarty, J.C., Watson, C.E., 1993. F₂ hybrids of host plant germplasm and cotton cultivars: I. Heterosis and combining ability for lint yield and yield components. *Crop Sci.* 33 (4), 700–705. <https://doi.org/10.2135/cropsci1993.0011183X003300040012x>.
- Taufiq Hidayat, R.S., Nurindah, D.A.S., 2019. Potential of brown cotton fiber development for sustainable textile materials. In: *Proc. of the Indonesian Textile Conference: textile 4.0 clothing and beyond (Int. Conference)*, (Vol. 3. Penerbit Politeknik STTT Bandung, p. 60.
- Tausif, K. 2008. Genetic studies on improving productivity and quality traits involving interspecific (H × B) crosses and barbadense genotypes (Master's thesis, University of Agr. Sci., Dept. of Genet. and Plant Breeding, College of Agriculture, India).
- Tu, L., Tan, J., Guo, K., Li, Z., Zhang, X., 2014. Flavonoid pathway in cotton fiber development. *Sci. Sin. Vitae* 44, 758–765.
- Vanapariya, S.J., Faldu, G.O., Patel, R.R., Viradiya, B.R., Rana, V.B., 2022. Combining ability and gene action analysis in intraspecific hybrids of cotton (*Gossypium hirsutum* L.). *Plant Arch.* 24 (1), 1195–1202. <https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.1.166>.
- Wang, P., Dong, N., Wang, M., Sun, G., Jia, Y., Geng, X., Liu, M., Wang, W., Pan, Z., Yang, Q., Li, H., 2022. Introgression from *Gossypium hirsutum* is a driver for population divergence and genetic diversity in *Gossypium barbadense*. *Plant J.* 110 (3), 764–780. <https://doi.org/10.1111/tpj.15702>.
- Wen, T., Luo, W., Li, Y., Lin, Z., 2024. Advances and new insights in naturally colored cotton breeding and research. *Ind. Crops Prod.* 211, 118252 <https://doi.org/10.1016/j.indcrop.2024.118252>.
- Zheng, H., Zhou, X., Lv, M., Zhang, X., Yang, B., Ke, L., Zhou, W., Sun, Y., 2024. The synergistic effect of fiber quality by proanthocyanidins and lignins in cotton fibers. *Ind. Crops Prod.* 214, 118581 <https://doi.org/10.1016/j.indcrop.2024.118581>.