BREEDING AND GENETICS

Genetic Analysis of Fiber Color Using Segregations of Color Parameters in Cotton

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ABSTRACT

Fiber color in cotton is a genetically inherited trait resulting from the presence of pigments intermingled with cellulose. Electronic shade-matching instruments can be used to determine objectively the color of cotton fiber. The objectives of this study were to investigate brown to white fiber color segregation in the F_2 population of 'Carmen' (white fiber) x 'Devetüyü-176' (brown fiber) using an electronic shade-matching instrument (chromameter) and compare those results to visual color analysis. The results demonstrated that transgressive segregations were identified for all fiber color parameters in the F_2 population. The fiber color parameters (L, ΔL, a, b, Δa, Δb, and ΔE) and the visual analysis were considered separately. Each parameter appeared to be controlled by a different single gene displaying complete or partial dominance of the brown color over white except L and ΔL. In summary, the results obtained from the use of the chromameter will aid in the reproducible and quantifiable determination of cotton fiber color and reduce observation errors inherent in visual analysis. This would be most useful in colored cotton breeding programs and in selection and in the monitoring of color drift.

C otton (*Gossypium hirsutum* L.) is one of the most important plants worldwide because of its fiber, which is used as a material for the textile industry. It is grown in many areas throughout the world. Most cotton cultivars in use have white fiber, consequently dyeing is required to obtain colored textiles (Hua et al., 2007).

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Naturally colored cottons are mutants of white fiber and there are several lint colors such as brown, black, red, pink, blue, and green (Hua et al., 2007; Khan et al., 2010; Murthy, 2001). Brown is the most common color and shades vary from light brown to intense mahogany red in the four cultivated as well as many of the wild species (Singh et al., 2000).

Fiber color is a genetically inherited characteristic resulting from the presence of pigments intermingled with cellulose in cotton (Dickerson et al., 1999; Carvalho et al., 2014). Kohel (1985) tested known genes for lint color for allelism and reported that the brown fiber color was controlled by six loci: Lc1 and Lc2 were responsible for medium brown color of lint, Lc3 was responsible for dark brown, and Lc4, Lc5 and Lc6 were responsible for light brown (Wang et al., 2014). Harland (1935) concluded from his crossing studies that brown lint color was controlled by different genes in *G. barbadense* L. and *G. hirsutum* L.

Although most cotton cultivars have white fiber, the inheritance of colored fiber has been the subject of several studies. Murthy (2001) stated that the genes responsible for fiber color often had pleiotropic effects, that is, controlling more than one trait. The fiber color of brown cotton was reported to be controlled by incomplete dominance of a major gene and an extra, recessive minor gene (Wu et al., 2010). Other research determined the inheritance of brown cotton fiber was controlled by a single incompletely dominant gene (Li et al., 2004; Zhan et al., 2008). Carvalho et al. (2014) crossed *G. barbadense* accessions representing different shades of brown and white *G. hirsutum* cultivars and concluded that all fiber colors were governed by a single gene. In contrast, using computer assisted identification, Zhu and Wang (2003) indicated that cotton fiber color is a quantitative trait controlled by polygenes.

Cuming et al. (2015) used electronic shade-matching instruments to determine color changes in cotton fiber. Instrumental color measurement readings are objective, quantifiable, and more rapidly obtainable compared to visual color deter-

mination (Karaarslan et al., 2013). Colorimeters and spectrophotometers are employed widely in commerce, industry, and laboratory to express color in numerical terms and to measure color differences between specimens. The main difference between a colorimeter and spectrophotometer is that a colorimeter is a device that measures absorbance of specific colors, whereas a spectrophotometer measures transmittance or reflectance as a function of wavelength (Randall, 2013).

The Commission Internationale de l'Eclairage Lab (CIE L*a*b, CIELAB) system has been used in determination of color differences. In this standard, L* represents lightness, a* represents the chromaticity coordinate for red-green (+a*: red direction, -a*: green direction), and b* represents the chromaticity coordinate for yellow-blue (+b*: yellow direction, -b*: blue direction). ΔL^*, Δa^*, and Δb^* are the difference in lightness and darkness, difference in red and green, and difference in yellow and blue, respectively. Within the CIE LAB color system, the color difference (ΔE) between two objects can be calculated according to the following equation: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Karaarslan et al., 2013; Ma et al., 2010).

The objectives of this study were to determine the color differences among the plants of an F_2 population constructed from cross between white x brown fiber genotypes using an electronic shade-matching instrument and to determine the inheritance of color parameters/changes.

MATERIALS AND METHODS

The cultivar, Carmen (*G. hirsutum*), was developed by Bayer Crop Science AG (Leverkusen, Germany) and has high yield potential. It has white fiber and meets the desired fiber quality parameters of the textile industry. 'Devetüyü-176' (*G. hirsutum*) originated from Pakistan and is a natural colored cotton line. It has brown fiber and the best fiber quality in the brown color scale.

The initial crosses between the parents Carmen and Devetüyü-176 were made in 2007. The manually selfed F_1 plants and F_2 generation were grown in the following 2 yr under field conditions. The seeds of all generations were sown by hand then thinned to 20 cm in 3-m rows. The width between the rows was 70 cm. The rows were sown at the experimental station of Ege University, Field Crops Department, Bornova, İzmir, Turkey. Fertilization

was applied as 80 kg/ha nitrogen and P_2O_5 before sowing, then 80 kg/ha nitrogen was given with both first and second irrigation. All field experiments were irrigated five times after flowering stage by furrow irrigation and the plants were hoed two times for weed control.

Harvestable bolls of the parents and 178 individual F_2 plants were picked twice by hand. The first harvest was made when 50% of the bolls found on the plants opened and harvested cotton was stored in the shade. In second harvest, cotton from individual plants was collected and combined with the first harvested cotton. The combined cotton was ginned with roller gin and color analyzed. No rain occurred during the cotton harvests. The fiber color of the individual F_2 plants were visually analyzed and classified as white or brown. In visual analysis, only the plants with white fiber color were recorded as white, all other shades of brown, even cream, were registered as brown. Fiber color of the parents (30 samples each) and each individual F_2 plant was quantified using L, a, b, ΔL, Δa, Δb, and ΔE parameters using a Minolta C400 Chromameter (Konica Minolta Holdings, Inc., Tokyo, Japan), an electronic shade-matching instrument. The fiber color results from visual and chromameter were analyzed using the Student's t-test and chi-square test (χ^2) in Microsoft Excel.

RESULTS AND DISCUSSION

The variation of the fiber color parameters from the parental genotypes and F_2 population are summarized in Table 1. The parental genotypes were significantly different from each other for all color parameters according to t-test values. The mean, minimum, maximum, and variance values of the color parameters in F_2 population give information about the color distribution of the population (Table 1). Transgressive segregations were identified for all fiber color parameters in the F_2 population. Cuming et al. (2015) used a chromameter to quantify color parameters and found transgressive segregations for all parameters except for ΔL in an F_2 population obtained from the cross of 'Yeşil' x 'Nazilli-84'. The frequency distribution graphs of each fiber color parameters in F_2 population can be followed from Fig. 1. There are continuous variations in the F_2 population for each color parameter (Fig. 1).

Table 1. Phenotypic variation of the fiber color parameters in the parental genotypes Carmen and Devetüyü-176 and F$_2$ population

Fiber color parameters[z]	Carmen			Devetüyü-176				Variation in F$_2$ population			
	Mean	Range	Var.	Mean	Range	Var.	t value[y]	Mean	Min.	Max.	Var.
L	87.6	80.5/91.0	6.8	64.9	56.8/74.5	8.8	23.8**	80.1	58.4	91.5	67.1
a	-0.1	-0.5/0.4	0.1	7.6	4.7/11.7	2.2	-22.7**	2.4	-0.5	7.9	6.4
b	6.7	4.2/8.9	1.6	22.1	17.7/30.4	6.3	-25.9**	13.5	5.1	24.6	29.9
ΔL	-5.7	-12.7/-2.2	7.1	-30.1	-36.9/-18.8	11.6	25.8**	-13.3	-34.8	-1.8	67.8
Δa	-0.4	-0.8/0.1	0.1	7.4	4.4/11.4	1.9	-23.1**	2.2	-1.0	7.6	6.4
Δb	8.9	6.3/11.1	1.6	22.9	16.3/32.6	10.7	-18.4**	15.7	7.2	27.7	30.6
ΔE	10.1	8.1/12.5	1.8	38.6	28.9/47.8	18.8	-29.7**	20.9	8.1	40.7	91.8

[z] Fiber color parameters: L indicates color brightness (L = 0 is black and L = 100 is white); a indicates red-green (+ is red and - is green); b indicates yellow-blue (+ is yellow and - is blue); ΔL indicates total black-white color change; Δa indicates total red-green color change; Δb indicates total yellow-blue color change; ΔE indicates total color change independent from each other.
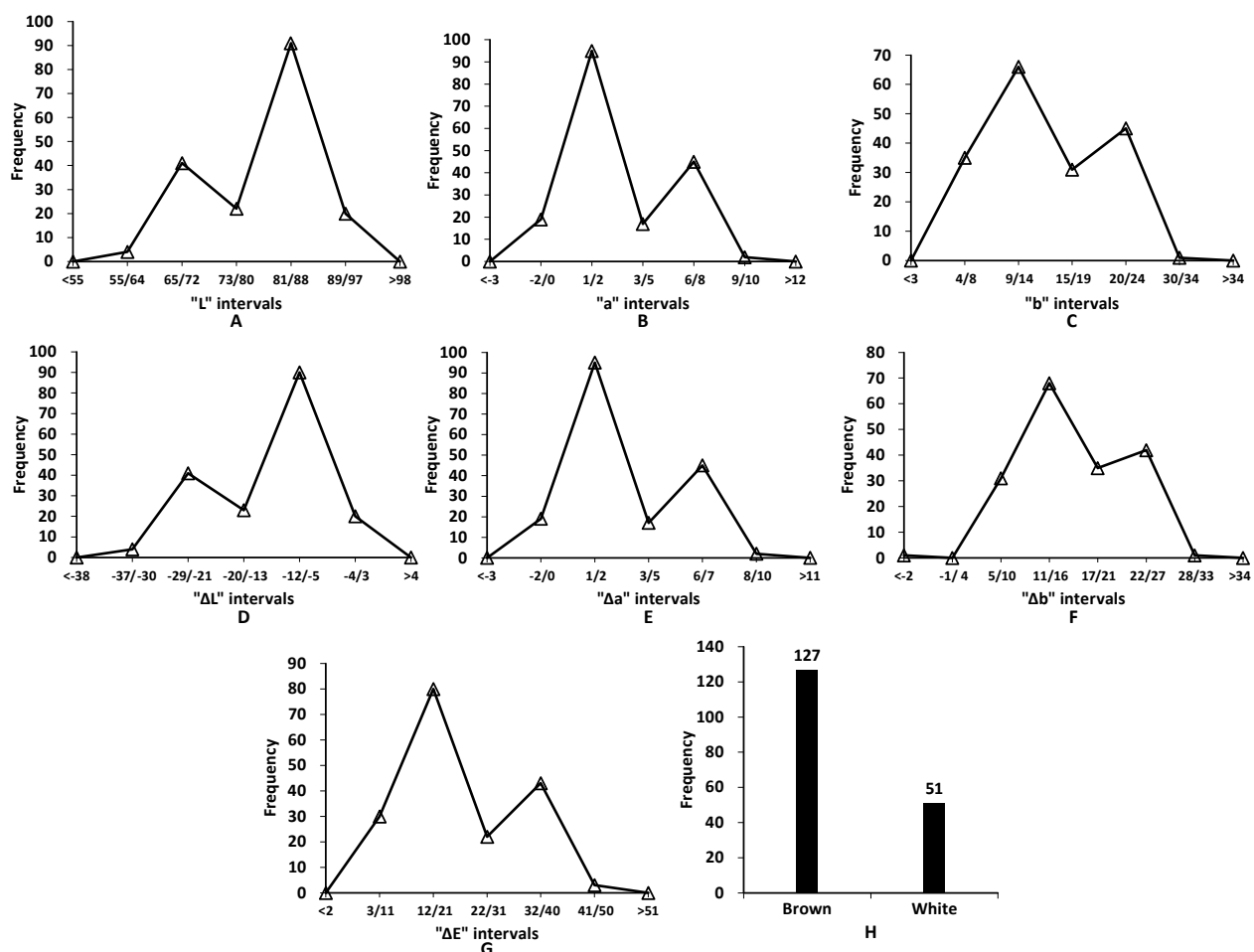
[y] Significant at p ≤ 0.01.

Figure 1. Frequency distribution of color parameters L, a, b, ΔL, Δa, Δb, ΔE and white-brown ratio in the F$_2$ population derived from the cross Carmen x Devetüyü-176.

The fiber color parameters of the parental genotypes were quantified in 30 samples for each parent. Hence, the ranges of measured color parameters of the parents (Table 1) were considered to create the phenotypic classes for each parameter in the F_2 population. The observed and expected ratios of phenotypic classes for each fiber color parameters are presented in Table 2. Among the 178 F_2 individuals from the Carmen x Devetüyü-176 cross, 129 individuals were identified as homozygous or heterozygous white and 49 were homozygous brown for the L parameter. Similarly, 127 individuals were determined as homozygous or heterozygous white and 51 were homozygous brown for the ΔL parameter. According to chi-square analysis, these results demonstrated that F_2 individuals segregated phenotypically in a 3 white:1 brown ratio ($\chi^2 = 0.61$, $p = 0.45$ for L, $\chi^2 = 1.27$, $p = 0.26$ for ΔL) (Table 2). Both distributions fit the inheritance model of a single dominant gene.

Less bimodal F_2 population distributions were observed for the other fiber color parameters (Table 2, Figs. 1B, C, E, F, G), which suggest that dominance was incomplete. The parameters a and b segregated in 52:75:51 and 48:77:53 ratios respectively, indicating 1 homozygous white:2 heterozygous:1 homozygous brown (Table 2). The chi-square analysis showed that these segregations fit the 1:2:1 expected ratio ($\chi^2 = 4.41$, $p = 0.10$ for a, $\chi^2 = 3.52$, $p = 0.18$ for b). Similarly, the observed ratios of the parameters Δa and Δb fit the 1:2:1 expected segregation ratio (51:76:51, $\chi^2 = 2.80$, $p = 0.24$ for Δa, 46:84:48, $\chi^2 = 0.50$, $p = 0.77$ for Δb). Total color change, ΔE segregated in 45 homozygous white:82 heterozygous:51 homozygous brown, which fit the 1:2:1 expected segregation ratio ($\chi^2 = 1.51$, $p = 0.47$). The inheritance patterns of a, b,

Δa, Δb and ΔE are described, therefore, as expressing incomplete dominance between two contrasting alleles. For these chromameter parameters, the allele for brown fiber color is incompletely dominant over the allele for white fiber color. Carvalho et al. (2014) reported similar results and concluded that fiber color is controlled by one gene with partial dominance of the brown color over white. Similarly, Wang et al. (2012) stated that brown fiber color was determined by single nuclear gene mainly presenting dominant heredity.

The results of visual analysis in F_2 population showed that fiber colors of 51 F_2 individuals were white and 127 individuals were brown, which fit a 1:3 segregation ratio (Table 2, Fig. 1H). These results of visual analysis are in agreement with findings of fiber color parameters except L and ΔL. In this study the fiber color parameters and the visual analysis were considered separately and each one appeared to be controlled by a different single gene. However, the inheritance models of L and ΔL parameters and the visual analysis are in accordance with complete dominance, which is white color over brown for L and ΔL parameters and the brown color over white for the visual analysis (Fig. 1A, D, H). In addition, the inheritance models of a, b, Δa, Δb, and ΔE parameters are described as partial dominance with brown color over white (Table 2). Each parameter is responsible for different colors and the formation of the fiber color in cotton is caused by the combination of different values of these parameters. Therefore, it is impossible to expect the same segregations or inheritance model from all color parameters in a F_2 population. In addition, the brown fiber color in cotton was revealed to be controlled by more than six loci, each of which is responsible for a different shade of brown (Kohel, 1985).

Table 2. Phenotypic segregation ratios and chi-square analysis of fiber color parameters among 178 F_2 plants derived from the cross Carmen (white) and Devetüyü-176 (brown)

Fiber color parameters	Observed ratio White:intermediate:brown	Expected ratio	χ^{2z}	p value
L[y]	129:49	133.5:44.5	0.61	0.45
a[x]	52:75:51	44.5:89:44.5	4.41	0.10
b[x]	48:77:53	44.5:89:44.5	3.52	0.18
ΔL[y]	127:51	133.5:44.5	1.27	0.26
Δa[x]	51:76:51	44.5:89:44.5	2.80	0.24
Δb[x]	46:84:48	44.5:89:44.5	0.50	0.77
ΔE[x]	45:82:51	44.5:89:44.5	1.51	0.47
white:brown[y]	51:127	44.5:133.5	1.27	0.26

[z] Significance limit of χ^2 ($p = 0.05$, df = 1 for 1:3 or 3:1, df = 2 in for 1:2:1) = 3.84 and 5.99, respectively.

[y] The values were relying on a 3 (including homozygous and heterozygous) : 1 expected Mendelian phenotypic segregation ratio for a single dominant gene.

[x] The values were relying on 1 homozygous : 2 heterozygous : 1 homozygous expected Mendelian genotypic segregation ratio for a single dominant gene.

This study is the first to use an electronic shade-matching instrument, the chromameter, to investigate the brown-white color distribution and to compare the results to the visual color analysis in an F_2 population in cotton. Zhu and Wang (2003) used an RGB (red, green, blue) analysis for the genetic dissection of colored cotton fiber in an F_2 population and commented that, compared to visual analysis, it offered the advantages of impersonality, repetitiousness, and reference ability. In this study, both the chromameter and visual methods demonstrated that each color parameter or visual color is controlled by a different single gene displaying complete or partial dominance. In summary, the results obtained from the use of the chromameter will aid in the reproducible and quantifiable determination of cotton fiber color and reduce observation errors inherent in visual analysis. This would be useful in colored cotton breeding programs and in selection and in the monitoring of color drift.

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