

## Patterns of color formation in different fibers during development of colored cotton (*Gossypium hirsutum* L.) cultivars

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### Abstract

We analyzed color development in four cotton (*Gossypium hirsutum* L.) cultivars, brown cotton (ZX-1 and XC-1), green cotton (4560 and G-7), and compared color parameters to white cotton (LMY28). Color value, chroma (Cab\*), and color difference ( $\Delta E_{ab}^*$ ) were studied by tristimulus colorimetry during fiber development. Results showed that lightness ( $L^*$  values) dimmed gradually and differences were significant ( $F=25.86-1575.18$ ,  $P<0.0001$ ) at the same developmental stage, while chromaticity ( $a^*$  values and  $b^*$  values) increased and showed an obvious peak. In addition, there were significantly different between cultivars during fiber development ( $F=40.12-13435.2$ ,  $P<0.0001$ ). The Cab\* of brown fiber increased slowly from 25 to 35 days post anthesis (DPA), deepened rapidly from 35 to 40 DPA, and from 55 DPA to maturation. The chroma of the XC-1 brown cotton cultivar was significantly higher than ZX-1 ( $F=130.36$ ,  $P<0.0001$ ) at maturation. The Cab\* of green fiber gradually deepened from 25 to 45 DPA, reached a maxima at 45 DPA. The Cab\* values of the G-7 green cotton cultivar were significantly higher than 4560 ( $F=46.28-4263.04$ ,  $P<0.0001$ ) during development. The  $\Delta E_{ab}^*$  values of brown fiber were small before 35 DPA, rose rapidly from 35 to 40 DPA, and rose rapidly again sharply with maturation ( $F=182.95$ ,  $P<0.0001$ ). The  $\Delta E_{ab}^*$  of green fiber increased rapidly, then slowly, but the rapidly increasing phases were different between cultivars, as the  $\Delta E_{ab}^*$  of G-7 fiber rose rapidly from 35 to 40 DPA, while that of 4560 fiber rose most rapidly from 30 to 35 DPA. The final  $\Delta E_{ab}^*$  of the 4560 cultivar reached a stable level of 12.84 at maturation, which was significantly lower than G-7 ( $F=4556.97$ ,  $P<0.0001$ ). Thus, the fiber color of brown cultivars gradually deepened before 35 DPA, rapidly deepened from 35 to 40 DPA, and from 55 DPA to maturation, while green cultivars gradually deepened from 25 to 45 DPA, reached a maxima at 45 DPA, and then faded from 55 DPA to maturation.

**Keywords:** Colored cotton; Color value; Chroma; Color difference; Tristimulus colorimeter.

**Abbreviations:** CIE: International Commission on Illumination; DPA: Days post anthesis.

### Introduction

Colored cotton (*Gossypium hirsutum* L.) is pigmented with different colors of which brown and green are the most common fiber colors. Colored fiber has the same structures as white fiber, including a similar primary wall, secondary wall, and lumen. Pigment is deposited in the lumen of brown fibers, but deposited in the lumen and inner secondary wall green fibers (Li et al., 2002; Zhang et al., 2011). Colored fibers are eliminate the need for dyeing in the fabric manufacturing process, and so are environmentally friendly, economical, and beneficial to human health compared to the conventional white cotton (Zhu et al., 2006). There are still many drawbacks, however, such as the poor quality and the instability of fiber pigment, which limits the further development of colored cotton (Wang et al., 2002; Dong et al., 2004; Li et al., 2004; Qiu et al., 2002). Wang et al. (2002) found that brown fibers significantly deepened in color from 30 to 35 DPA, and then became deeper and deeper in the sunshine. In contrast, green fibers appeared at 20 DPA, then deepened gradually, but faded in the sun. Dong et al. (2002) reported that fiber color formed at mid-late developmental stages. Colored cotton fibers were white before primary wall stretching, and appeared very light in color until secondary wall thickening (Qiu et al., 2002). However, Waghmare et al. (1998) found that the sun was necessary for the development of color after opening of bolls,

but that the color would fade when exposed to continuous sunshine. Fiber quality is negatively correlated with color differences ( $\Delta E_{ab}^*$ ) (Li et al., 2004) and chromaticity (Chen et al., 2001), so it is essential to study the pattern of color formation during fiber development to determine the best quality fibers for manufacturing. To this end, we used tristimulus colorimetry to study the parameters of fiber color during development and at maturation.

### Results

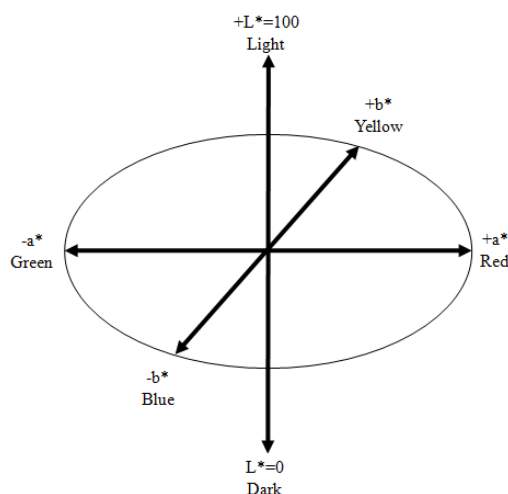
#### *Dynamic changes in color values of different fiber during development*

The  $L^*$ ,  $a^*$ ,  $b^*$  values were recorded (Table 1) and used to calculated other color parameters, including Cab\* and  $\Delta E_{ab}^*$ . These data were used to establish the utility of the colorimetric method. As given in the table, mean  $L^*$  values for white cotton were higher than those for colored cotton cultivars. The mean  $L^*$  values decreased gradually with fiber development, and there were significant differences among colored cotton cultivars ( $F=25.8-1575.18$ ,  $P<0.0001$ ). The mean  $L^*$  value for LMY28 reached a maximum of 105.71 at 35 DPA. After 55

**Table 1.** Dynamic changes in color values of different fiber during development

Cultivars	Color value *	Days post anthesis (d)							Maturation
		25	30	35	40	45	50	55	
LMY28	L*	104.16	103.65	105.71	96.45	97.06	100.94	100.65	97.10
	a*	-4.94	-4.52	-5.75	-0.31	-0.06	-1.64	-1.69	-0.06
	b*	-0.80	-0.82	1.26	6.42	5.99	4.03	2.30	1.90
ZX-1	L*	95.49	94.87	94.89	87.48	88.51	88.81	88.12	83.06
	a*	0.01	0.54	0.23	5.00	3.92	3.64	3.36	3.41
	b*	2.34	2.91	2.95	7.20	9.16	8.34	9.32	12.46
XC-1	L*	96.79	96.66	97.02	88.23	90.29	89.37	88.12	81.72
	a*	-0.06	-0.17	0.10	4.45	3.61	3.87	3.38	4.25
	b*	2.14	2.66	1.96	6.57	7.09	8.14	9.20	14.14
G-7	L*	103.90	98.75	96.75	86.44	87.56	85.17	83.89	79.74
	a*	-5.94	-7.17	-7.57	-2.04	-2.90	-3.12	-3.57	-2.46
	b*	1.11	2.51	7.45	12.78	16.37	15.45	15.27	12.63
4560	L*	102.00	103.43	94.86	94.37	94.96	94.62	92.99	91.15
	a*	-4.94	-4.67	-0.22	-0.47	-1.95	-1.65	-1.97	-1.52
	b*	1.73	3.61	6.39	7.09	7.98	8.61	7.87	7.64

\* L\* indicated white-black; a\* indicated redness-greenness; b\* indicated yellowness –blueness; maturation indicated opening of bolls.



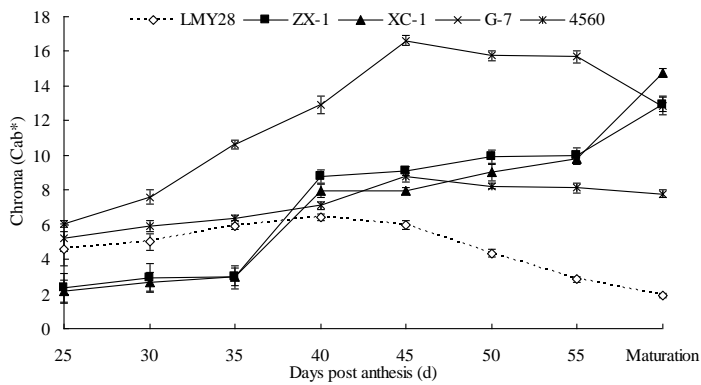
**Fig 1.** CIELAB color space. Color can be described by two factors: the brightness of the color (chroma; the brighter the color, the higher the chroma) and the darkness or lightness of the color (the L\* value; black=0 and white=100). The a\* (value between 60=red and -60=green) and b\* (value between 60=yellow and -60=blue) are the ground values for calculating chroma.

DPA, L\* gradually decreased to 97.10 at maturation. The mean L\* value for white cotton was significant higher ( $F=3251.82$ ,  $P<0.0001$ ) than colored cotton cultivars. The a\* values, which represent the degree of red-green within the color space, increased with fiber development and exhibited periods of more rapid increase. Moreover, there were significant differences among colored cotton cultivars ( $F=931.22-13435.2$ ,  $P<0.0001$ ). The a\* values of ZX-1 and XC-1 increased in parallel; a\* values were at or near the origin of coordinates (0, 0) before 35 DPA, increased rapidly to 4.45–5.00 at 40 DPA, then gradually decreased. The a\* values of G-7 and 4560 were negative (green), and the a\* value of G-7 was lower than 4560 during the same developmental stage. The a\* values of LMY28 were small before 35 DPA, increased to -0.06 at 45 DPA, and decreased again before rising to -0.06 at maturation. The b\* values, which represent the degree of blue-yellow within the color space, increased with fiber development and there were significant differences ( $F=40.12-6453.88$ ,  $P<0.0001$ ) among colored cotton cultivars at the same developmental stage. The ZX-1 and XC-1 cultivars showed few differences and exhibited similar trends in b\* values during development. The b\* values of both rapidly increased from 35 to 40 DPA, and from 55 DPA

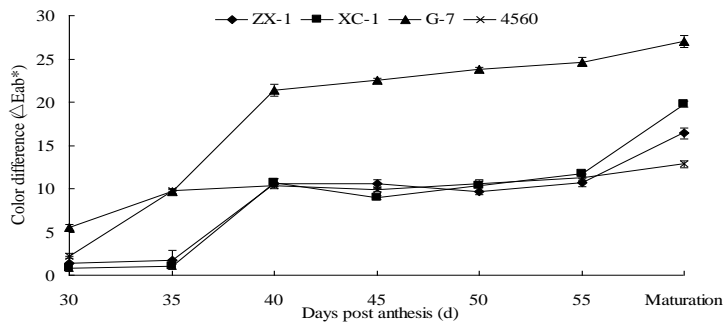
to maturation. The b\* values of G-7 and 4560 first increased, then declined, and were significantly different ( $F=6.93$ ,  $P=0.0250$ ) or extremely different ( $F=102.98-3793.54$ ,  $P<0.0001$ ) in every developmental stage. The b\* values of G-7 reached a maximum at 45 DPA, while 4560 peaked at 50 DPA. Both decreased thereafter. In contrast, the b\* values of LMY28 was reached a maximum of 6.42 at 40 DPA, and then gradually reduced to 1.90 at maturation.

#### Dynamic chroma changes during development

The Cab\* values were calculated as described in the Materials and Methods section. The Cab\* values of colored cotton cultivars increased with fiber development as shown in Figure 2. For ZX-1 and XC-1 cultivars, Cab\* increased from 2.34 and 2.14 at 25 DPA to 9.96 and 9.81 at 55 DPA, then increased to 12.91 and 14.76 at maturation. Thus, the final Cab\* of XC-1 was higher than ZX-1 at maturation ( $F=130.36$ ,  $P<0.0001$ ). For G-7 and 4560, similar trends in Cab\* were observed; Cab\* gradually increased from 6.04 and 5.23 at 25 DPA to reach maxima 16.63 and 8.76 at 45 DPA, then declined to 12.86 and 7.79 at maturity. Thus, the Cab\* values of G-7 were significant



**Fig 2.** Dynamic changes of chroma during fiber development of different cotton cultivars. 35 DPA: the onset of quickly increasing chroma for ZX-1 and XC-1 cultivars; 25 DPA: the onset of the gradual increase in chroma for G-7 and 4560 cultivars and the peak at 45 DPA.



**Fig 3.** Dynamic changes in color difference between fibers during development of colored cotton cultivars. 35 DPA: the onset of quickly increasing color for ZX-1 and XC-1 cultivars; 30 DPA: the onset of increasing color for G-7 and 4560 cultivars.

greater than those for the 4560 cultivar during the entire period of fiber development ( $F=46.28-4263.04$ ,  $P<0.0001$ ). The  $Cab^*$  values for LMY28 reached a maximum of 6.43 at 40 DPA, then decreased gradually, and was significant lower than all colored cotton cultivars at maturation ( $F=2969.35$ ,  $P<0.0001$ ).

#### Dynamic changes of color difference during development

Figure 3 graphically illustrates the mean  $\Delta Eab^*$  values for each cultivar. The  $\Delta Eab^*$  values increased during development and there were significant differences between cultivars throughout ( $F=137.41-2513.89$ ,  $P<0.0001$ ). Cultivars ZX-1 and XC-1 exhibited similar increasing trends in  $\Delta Eab^*$ ;  $\Delta Eab^*$  were small before 35 DPA, rose from 1.75 and 0.99 at 35 DPA to 10.70 and 11.70 at 55 DPA, and raised again to 16.39 and 19.74 at maturation. At maturation, the  $\Delta Eab^*$  value of XC-1 was significant greater than that of ZX-1 ( $F=182.95$ ,  $P<0.0001$ ). In contrast, the  $\Delta Eab^*$  values of G-7 and 4560 increased rapidly and then slowly, but there were significant differences in the timing and magnitude of the rapidly increasing periods; specifically, the  $\Delta Eab^*$  values of G-7 were larger during development and at maturation ( $F=4556.97$ ,  $P<0.0001$ ), as  $\Delta Eab^*$  values increased sharply from 9.70 at 35 DPA to 21.36 at 40 DPA, then increased more slowly to 27.00 at maturation. In contrast, the  $\Delta Eab^*$  values of 4560 increased sharply from 2.23 at 30 DPA to 9.78 at 35 DPA, then increased slowly

to 12.84 at maturation. These development changes in fiber colors are presented in Figure 4.

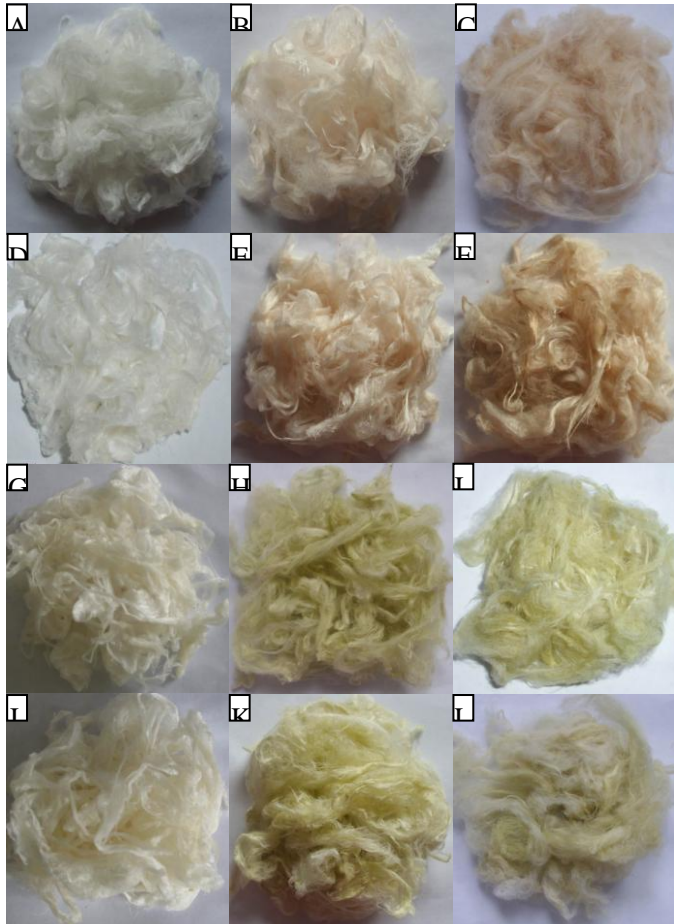
#### Discussion

Variations in color can be reflected by the changes in color values ( $L^*$  values,  $a^*$  values and  $b^*$  values),  $Cab^*$ , and  $\Delta Eab^*$  (Chan et al., 2001; Mendoza et al., 2006; Coralia et al., 2007; Schmitzer et al., 2009; Duane et al., 2000; Stavros et al., 1998; Akhtar et al., 2007). The results in this paper showed that the tested colored cotton cultivars had similar general patterns of color formation during development, but significant differences in rates and periods of increase and in final values at maturation ( $F=25.86-13435.2$ ,  $P<0.0001$ ). In the brown cultivars ZX-1 and XC-1,  $L^*$  values suddenly diminished,  $a^*$  values increased sharply in favor of red, and  $b^*$  values increased sharply in favor of yellow from 35 to 40 DPA. In contrast, there were differences in the timing of rapidly increasing periods between G-7 and 4560; In the green cultivar G-7,  $L^*$  values suddenly diminished and  $a^*$  values increased sharply in favor of green from 35 to 40 DPA, but  $b^*$  values increased sharply in favor of yellow from 30 to 35 DPA; while in 4560,  $L^*$  values suddenly diminished,  $a^*$  values increased sharply in favor of green, and  $b^*$  values increased sharply in favor of yellow from 30 to 35 DPA. These results indicated that fiber color deepen gradually with fiber development, but there were differences in the deepened periods of colored cotton cultivars. We noted several differences from previous studies (Qiu et al., 2002; Dong et al., 2002) and described the development of color patterns and formation in more detail by documenting multiple parameters. Different chemical extraction methods have been used to isolate the different components of cotton pigments, and these results revealed different chromogenic periods because of different compositions of pigments (Wang et al., 2002; Zhan et al., 2006). This research further indicated that changes in  $Cab^*$  and  $\Delta Eab^*$  values paralleled changes in chromaticity, while there were contrary changes in  $L^*$  values. The period of rapidly increasing  $Cab^*$  and  $\Delta Eab^*$  values in brown cotton occurred between 35 and 40 DPA, while the  $Cab^*$  values for green cotton cultivars showed no obvious rapidly increasing period. Rather,  $Cab^*$  grew gradually from 25 to 45 DPA, and decreased slightly at maturation. There were differences in  $\Delta Eab^*$  values between G-7 and 4560 but similar trends;  $\Delta Eab^*$  values of G-7 rose sharply from 35 to 40 DPA while the  $\Delta Eab^*$  values of 4560 grow more rapidly between 30 and 35 DPA. At maturation, the  $\Delta Eab^*$  value of 4560 was significant lower than that of G-7 ( $F=4556.97$ ,  $P<0.0001$ ). The results of this study showed that fiber colors increased with fiber development, with significant differences in timing between brown and green cultivars. Brown cotton rapidly increased in color from 35 to 40 DPA, and then increased slowly, while green color increased gradually, and achieved maxima at 45 DPA before decreasing. Our results are similar to Wang et al. (2002) but we show periods of brown color increase distinct from Zhan et al. (2006). We performed a detailed analysis of color formation in different fibers from a colorimetric angle and clearly demonstrate differences in these color changing processes between cultivars during development.

#### Materials and methods

##### Plant materials

These experiments were conducted during 2008–2009 at the Experimental Station of the College of Agriculture, Shandong Agricultural University, Taian, China. The cultivars studied were brown cotton (ZX-1 and XC-1) and green cotton (G-7 and



**Fig 4.** Pictures of fiber color during development. A–C and D–F show fiber colors of ZX-1 and XC-1 cultivars at 35 DPA, 40 DPA, and maturation. G–I and J–L show fiber colors of G-7 and 4560 cultivars at 25 DPA, 45 DPA, and maturation.

4560), with white cotton (LMY28) as the control. The ZX-1 and 4560 cultivars were obtained from Institute of Cotton Research, Chinese Academy of Agricultural Sciences. The XC-1 and G-7 cultivars were obtained from Xinjiang Tiancai Technology Co. Ltd and Gansu Academy of Agricultural Sciences Crop Research, respectively. The cultivars were planted in a randomized block design with three field replications while maintaining row to row and plant to plant distances of 80 and 30 cm, respectively. The soil contained 11.59 g kg<sup>-1</sup> organic matter, 66.22 mg kg<sup>-1</sup> alkali-hydrolysable N, 33.34 mg kg<sup>-1</sup> available P, and 79.03 mg kg<sup>-1</sup> available K. Cotton flowers were tagged on the day of anthesis to allow harvesting of bolls of known ages. Tagged bolls (fruit) were harvested first at 25 DPA, and then were harvested at 5-d intervals during the course of fiber maturation. Each harvest consisted of 4–8 bolls from each of the three cultivar replications. Fibers were separated from the ovules, and then naturally air-dried in the shade before colorimetry.

#### Measurements

Fiber color was measured using a tristimulus colorimeter equipped with a CR-300 measuring head. The instrument was calibrated with a white standard calibration plate before use. The color measurement can be described according to the CIE (1976) L\*a\*b\* (CIELAB) color space values (Judd and

Wyszecki, 1975; Bilmeyer and Saltzman, 1981; Hunter and Harold, 1987) (Fig. 1).

#### Color value measurement

In this system, the L\* value corresponds to a dark–bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). The a\* and b\* values extend from -60 to +60; a\* negative is green and positive is red; b\* negative is blue and positive is yellow (Schmitzer et al., 2009); The origin of the coordinates, a\* = 0, b\* = 0, indicates no color. The L\*, a\*, b\* values of every developmental stage of each cotton cultivar were measured 10 times and averaged.

#### Chroma measurement

Chroma (Cab\*) expresses the degree of color for an area viewed in relation to its brightness, and can be estimated by  $Cab^* = (a^{*2} + b^{*2})^{1/2}$  (McGuire, 1992). Chroma (Cab\*) is larger when color coordinates are farther from the origin.

#### Color difference measurement

The total color difference ( $\Delta Eab^*$ ) is a relational index between standard color and sample color in colored space. This study used 25 DPA fibers as control samples to calculate the color differences during development. The mean values of  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  after 10 measurements were automatically calculated by the colorimeter and recorded. The  $\Delta Eab^*$  was calculated from the mean  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  values; that is,  $\Delta Eab^*$  between any two points in the color space can be obtained from the following correlation:  $\Delta Eab^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$  (Wyszecki G, 1982).

#### Statistical analyses

The color values (L\* values, a\* values and b\* values), as well as Cab\* and  $\Delta Eab^*$  were processed using Microsoft Excel 2003 and Statistical Analysis System (SAS, V. 8.1, SAS Institute, Cary, NC). Means of changes in color values, Cab\*, and  $\Delta Eab^*$  were compared among the ZX-1, XC-1, G-7, 4560, and LMY28 cultivars using Duncan's test. A P < 0.05 was regarded as statistically significant.

#### Conclusion

The results presented here revealed that brown fibers from ZX-1 and XC-1 cultivars deepened gradually before 35 DPA, then deepened rapidly from 35 to 40 DPA, and from 55 DPA to maturation, while green fibers from G-7 and 4560 cultivars showed gradually increasing color from 25 to 45 DPA, reaching the maxima at 45 DPA before fading from 55 DPA to maturation.

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