# ORIGIN AND GROWTH OF EXTINCTION BANDS IN COTTON

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## Abstract

In this paper, the literature on, the origin and growth of reversal extinction bands in cotton fibres, as observed along their lengths in crossed polarized light in a polarizing light microscope, has been briefly reviewed and extensive data on the frequency of reversal/extinction bands in field matured fibres of a large number of the same cotton varieties, grown at four different locations of growth in India, are presented and discussed. It is observed that the frequency of reversal/extinction bands per centimeter length of cotton fibres for the same varieties grown at different locations remain practically invariant, although their value varies between individual varieties. Apparently, reversals/extinction bands observed along the length of cotton fibres,appear to be genetically determined.

## Introduction

Cotton fibre is about 93-96% pure cellulose and this cellulose has a fibrillar structure. A bulk of this cellulose is laid in the secondary diurnal growth layers during development of the fibre inside the cotton boll ( Bowmann, 1908; Balls, 1919, 1928; Berkeley, 1942; Warwicker et.al., 1966; Barrow, 1940; Frey Wyssling, 1954; Preston, 1957; Westafer and Brown, 1976). The secondary diurnal growth layers have been shown to arise as a result of temperature cycles (Balls, 1919 ; Denham, 1922; Roelofsen, 1951; Stove,1957; Dlugosz, 1965; Berrimam, 1966; Grant et.al., 1966; Eagle and Grant, 1970; Grant et.al., 1970 and 1972; Stephens, 1978; Anderson and Kerr, 1938). Cellulose fibrils in the individual secondary layers ,form a spiral about the fibre axis (Bowmann, 1908; ,Balls, 1923, 1928; Warwicker et.al., 1966; Preston, 1952; Nanjundayya, et.al., 1960; Rebenfeld, 1965). The orientation of these fibrils in the successive secondary layers is believed to be all left handed, alternating between left and right handed, whereas some workers believe that the sense of the helix within individual secondary layers deposited, reverses several times along the length of the fibre (Bowmann, 1908; Balls, 1919, 1928; Nanjundayya et.al., 1960; Rebenfeld, 1965 Muhlethaler, 1949; Kerr, 1946; Rollins et.al., 1958; Tripp et.al., 1960; Hesketh and Low, 1968; Porter et.al., 1960). These so called reversal points are identified as dark extinction bands in a polarizing light optical microscope under crossed polarized field. Balls, 1923 and Balls and Hancock, 1956, proposed for the first time that the longitudinal spirals of micro-macro fibrillar (for optical microscopy) cellulose within secondary diurnal growth layers of cotton, reverse their sense of orientation several times along the length of the fibres, and in the process come into a configuration where the crystallites lie parallel to the fibre axis in a very narrow region and thereby exhibit an extinction band under crossed polarized light. Balls called them as "Reversals" and ever since then, everyone has called them so. It may be mentioned that Balls had made these observations when many high resolution instrumental techniques, such as the transmission and scanning electron microscopes, had not even been invented. Each of these new techniques in subsequent investigations as applied to cotton using ultrasonically disintegrated fragments of secondary layers of cotton fibres by negative and positive staining , shadow casting techniques and by carbon surface and freeze fractured replicas have however not shown any clear evidence of the existence of an abrupt change in orientation in secondary layers (Dlugosz, 1965; Muhlethaler ,1949; Willison and Brown, 1972; O Connors, 1973; Kolpack and Blackwell; 1975; Moharir, 1980; Manley, 1964; Rollins et.al., 1972). Parallelly-laid cellulose fibrils only are however seen. Some evidence of cross-orientations in successive secondary layers deposited in definite wave patterns in developing cotton fibres have been shown by freeze-fractured replica technique in a transmission electron microscope (Willison and Brown, 1972). The evidence in respect of the hypothesis of reversibility of cellulose fibrils in secondary growth layers, therefore still continues to be derived only from the polarizing microscope and at the optical miscroscopic level of resolution, although not without doubts (Roelofsen, 1950, 1951, 1954; Iyengar, 1964; Rowland et.al., 1976). Reversals of cellulose fibrils in individual secondary growth layers are believed by some (Raes et.al., 1968; Viswanath, 1975) to coincide exactly or nearly so. If however true, the entire number of extinction bands should then be generated in the very first secondary layer deposited in a developing cotton fibre. Practical observations however indicate that the number of extinction bands increase with increase in the number of secondary layers deposited and consequently with the maturity of the fibres. Moreover, the intensity of blackening of extinction bands should progressively increase with the superimposed deposition of each secondary growth layer. No such report exists. In a system, like the cotton, where internal dimensions are changing with each deposition of secondary growth layer (Hessler, 1961; Waterkeyn et.al., 1975), when the degree of polymerization of cellulose reported to be remaining constant throughout the cotton developing phase, more particularly, because of the fact, that the secondary wall thickness within developing cotton fibres is not being uniformly thick along its vertical profiles (Peeters et.al., 1987), such a possibility of exact superposition of reversals within successive individual secondary growth layers seems doubtful.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1583-1587 (1998)
National Cotton Council, Memphis TN

Extinction bands have been mapped by several investigators (Eagle and Grant, 1970; Kolpack and Blackwell, 1978; Herbert and Boylston, 1984; Orr et.al., 1961; Morosoff and Ingram, 1970; Iyer, 1965; Fransen and Verscraege, 1967; De Boer, 1978), and their frequency is reported to be influenced by genetic (Raes et.al., 1968; Fransen and Verscraege, 1967; De Boer, 1978; Clegg and Harland, 1924; Betrabet et.al, 1965), and environmental factors (Roelofsen,1951 ; Stove, 1957; Dlugosz, 1965; Moharir, 1980; Fransen and Verscraege, 1967; Betrabet et.al., 1965; Moharir et.al., 1979) and by irrigation (Fransen and Verschraege, 1967). The number of extinction bands have been shown to be more in tetraploid *Gossypium barbadense* and *Gossypium hirsutum* than in diploid *Gossypium herbaceum* and *Gossypium arboreum* varieties of cotton (Balls, 1923; De Boer, 1978; Hoch, 1952; Betrabet et.al., 1963; Maursberger, 1954; Pillai, 1961). Raes et.al., 1968 have proposed a mathematical model for the distribution of extinction bands and they conclude that the fibre presumably consists of sequences of elementary units more or less constant in length, which are responsible for the observed pattern in the reversal distance distribution. At the same time, it is almost clear that the reversals are actually formed during the secondary thickening period, in a developing cotton fibre and their number, progressively increases as the secondary thickening and maturity proceeds. A definite correlation between the number of extinction bands and secondary growth layers in a developing cotton fibre has been seen. Wide variations in inter-reversal band distributions have been reported for cotton of different origin and species. They are reported to be much more frequent at the tip end of the fibre than at the base end. Their distribution from independent studies, has been confirmed to be of a log-normal type and implies that there is a minimum periodic distance between reversals/extinction bands. Positions of extinction bands are again shown to be weak points by some workers (Wakeham and Spicer, 1951, 1955; Warwicker et.al. 1970; Raes et.al., 1969, 1971; Fransen and Verschraege, 1970; Raes et.al. 1971), but as strong points by some others (Warwicker et.al., 1970; Mason, 1959; Wakeham et.al., 1959; Viswanath 1975; Hebert 1979; Hearle and Sparrow 1971). If reversals are really weak points, their increased number in *Gossypium barbadense* varieties ( Raes et.al., 1968; Betrabet et.al., 1963) cannot be easily reconciled with higher tensile strengths of the fibres of this species (De Boer, 1978; Maursberger, 1954; Wakeham et.al., 1959). A more recent interpretation of this phenomenon indicates that the reversals may be strong but because of its existence in the fibre, stresses occur adjacent to it, causing the ultimate fracture (Wakeham and Spicer,1955). This approach is compromised from the Scanning Electron Microscope/ Fibre fractography study of Hearle and Sparrow, 1971, which shows that the fracture of cotton fibre occurs in the reversal zone area but not at the precise reversal point. Other methods have also implied the same conclusion (Hebert,1979). Raes et.al., 1968 ; have sounded a well considered caution on the basis of their own results and say that it is necessary to be very careful in the interpretation of data concerning breaks "at" and breaks "between" reversals.

Waterkeyn and others (Anderson and Kerr, 1938; Muhlethaler, 1949 ; Waterkeyn, 1985; Ising, 1965; Dlugosz, 1965) have classified reversals of two kinds "crossed " and "bent" types. Close observations of extinction bands in crossed polarized light (Moharir et.al., 1986), indicate that every extinction band alternates from dark to bright at each 20-27 $^{o}$ rotation of the stage of the microscope. Indicating thereby the presence of two sets of cross oriented crystallized matrix of cellulose fibrils at the extinction band position. This observation is also strengthened by the fact that the equatorial (002) reflection in the X-ray diffractogram of single cotton fibre taken at the extinction band position is not a mere Laue spot, but a diffused crescent, and this diffusion though small, is essentially taken as a measure of very high crystallinity and orientation of cellulose crystallites to the fibre axis at these band positions.

Several attempts have been made over the years to explain the origin and growth of extinction bands and yet the exact cause and manner are not definitely known (Balls,1928; Grant et.al.,1966; Iyengar,1964). Moharir et.al.,1986,1992; reviewing the literature on the origin and growth of extinction bands, employing several techniques such as wide, small angle and microfocus X-ray diffraction, transmission and scanning electron microscopy, optical and polarized light microscopy etc., have explained the origin and growth of extinction bands, without resorting to the reversal hypothesis, on the basis of relative phase shifts in the deposition of microfibrillar cellulose within individual secondary diurnal layers of a developing cotton fibre. The origin of these bands according to Moharir et.al., 1986, 1992; is a phenomenon between secondary layers which may be discernible or not, because cotton fibres grown under constant temperature and illumination conditions without discernible secondary growth layers have also been reported to exhibit extinction bands and it is quite evident that they actually owe their generation to the pattern and manner in which the crystalline cellulose is deposited in space and dimensions in a developing cotton fibre and that too at the optical microscopic level of resolution.

Wakeham and Spicer, 1955; reported the reversal frequency to be genetic, peculiar to a cotton variety. Others ( Westafer and Brown, 1976; Hirsch and Jacquemart, 1953) found a connection between reversal frequency and fibre maturity and still others (Preston, 1957; De Coene and Verschraege, 1963; Fransen and Verschraege, 1968) found no such correlation. Hebert and Boyston, 1984; observed increase in reversal frequency with maturity of fibres from the day of antheses in five cotton cultivars and this frequency varied in each cotton variety though the extent of variation was very narrow.

In this report, data on reversal frequency, physically counted in mature cotton fibres of the same set of cotton cultivars grown at different locations and in different crop years in India are presented and discussed. It is believed that the observations reported would provide answers to many outstanding enigmatic questions about the reversals in cotton fibres.

## Materials and Methods

Mature, ginned,fibres of cotton cultivars grown at Sirsa, New Delhi, Nagpur and Coimbatore locations in India during 1992,1994 and 1995 were collected. The fibres were mounted parallel straight on glass slides with the help of quickfix adhesive and closely ruled sheet under the slide. The slides were mounted on Lietz polarizing microscope under crossed polarizer and analyzer conditions and the number of extinction bands were physically counted on each fibre along their lengths, moving them linearly. Measurements on 150 individual fibres for each variety were made and average value of reversals per unit centimeter length of fibre was computed. Further, location wise average values for each cotton variety for the three crop years 1992,1994 and 1995 were computed and the data are presented in Table 1.

## Results and Discussion

It may be observed from Table-1, column 4, that in general, the tetraploid cotton varieties of Gossypium hirsutum show slightly increased number of reversals/extinction bands per centimeter of fibre length as compared to the diploid Gossypium herbaceum and Gossypium arboreum cotton varieties. Consequently, the average inter-reversal distance for varieties of diploid species of cotton is higher as compared to that for the tetraploid varieties, Table-1, column 5. Further it may be observed from column 4, that the average value of the number of reversals per centimeter length of fibres within a variety almost practically remains constant irrespective of the location of growth of cotton. This is also evident from the lower values of the standard deviations. The data from the All India Coordinated Cotton Improvement Project ( Makwana and Iyer, 1996 ), indicates that the maturity of cotton fibres increases with increase in the latitude of the place of growth of cotton . And two extreme locations of cotton growth in the present study, namely Coimbatore and Sirsa lie between $11^o$ and $29^o$ North latitudes in India. This clearly brings out that the number of extinction bands per unit length of cotton fibres ,obviously does not depend upon the latitude of the place of growth of cotton and incidently upon the maturity of cotton fibres. This result is in conformity with the results reported earlier ( De Coene and Verschraege 1963). The average inter-reversal band distance, Table 1, Column 5 , further indicates that the distribution of reversal extinction bands along the length of cotton fibres within a variety is periodic in origin as is also suggested earlier by Raes et.al.1968; and Iyer,1965. And since this inter-reversal distance within a cotton variety remains practically invariant, irrespective of the location of growth of cotton ( Table 1, column 5), it is also concluded that they are genetic in origin as was concluded earlier by several other workers ( Raes et.al., 1968; Fransen and Verschraege, 1967; Clegg and Harland 1924; Betrabet et.al.,1965). To the best of our knowledge, this is the first major report on reversal frequency data in a large number of same cotton varieties grown at different agroclimatic locations in the literature and it is believed that this would finally help to set at rest the controversy about the reversals/extinction bands being genetic or environmental in their origin.

## Summary

Data in respect of a large number of the same cotton varieties grown at four different agroclimatic locations in India during 1992, 1994 and 1995 crop years indicate that the locationwise average frequency of reversals/extinction bands per unit length of fibres observed in crossed polarized light in a variety appears to be genetic in origin. Their value varies between different varieties but remains practically constant within individual varieties irrespective of the location of growth of cotton. Minor variations in the reversal frequency can be attributed to the manner in which the crystalline cellulose aggregates are actually formed within the secondary growth layers of developing cotton fibres, depending perhaps upon several complex environmental and local conditions of growth.

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**Acknowledgement**

The Authors thank the Commission of European Communities, Brussels, Belgium for the Project grants vide Contract No. CI *1 CT93-0077.

Table-1 Locationwise data on number of extinction bands per unit lenght of cotton fibers in diploid and tetraploid varieties.

| Cotton variety 1 | year of growth 2 | Location of growth 3 | extinction bands/cm 4 | Av.extinction band dist.(cm) 5 |
|---|---|---|---|---|
| Tetraploid varieties | | | | |
| B.N. | 1992 | Nagpur | 22.80 | 0.043 |
| B.N. | 1992 | Coimbatore | 20.36 | 0.049 |
| B.N. | 1994 | Sirsa | 22.97 | 0.043 |
| B.N. | 1994 | New Delhi | 19.65 | 0.050 |
| B.N. | 1995 | New Delhi | 18.45(L) | 0.054(H) |
| B.N. | 1995 | Nagpur | 21.83 | 0.045 |
| B.N. | 1995 | Coimbatore | 23.51(H) | 0.042(L) |
| Range within variety (H-L) | | | 5.06 | 0.012 |
| Average value | | | 21.36 | 0.046 |
| Standard Deviation | | | 1.91 | 0.004 |
| Suvin | 1995 | New Delhi | 22.70 | 0.044 |

| | | | | |
|---|---|---|---|---|
| LRA-5166 | 1994 | Sirsa | 20.78 | 0.048 |
| LRA-5166 | 1994 | New Delhi | 22.27 | 0.045 |
| LRA-5166 | 1994 | Nagpur | 21.35 | 0.046 |
| LRA-5166 | 1994 | Coimbatore | 22.19 | 0.045 |
| LRA-5166 | 1995 | New Delhi | 21.84 | 0.045 |
| LRA-5166 | 1995 | Nagpur | 18.89(L) | 0.053(H) |
| LRA-5166 | 1995 | Coimbatore | 22.74(H) | 0.044(L) |
| Range within variety (H-L) | | | 8.85 | 0.009 |
| Average value | | | 21.43 | 0.046 |
| Standard Deviation | | | 1.29 | 0.003 |
| AC-738 | 1994 | Sirsa | 20.40 | 0.049 |
| AC-738 | 1994 | New Delhi | 19.26(L) | 0.052(H) |
| AC-738 | 1994 | Nagpur | 20.88(H) | 0.047 |
| AC-738 | 1995 | Coimbatore | 19.35 | 0.051 |
| Range within variety (H-L) | | | 1.62 | 0.005 |
| Average value | | | 19.97 | 0.050 |
| Standard Deviation | | | 0.79 | 0.002 |
| Diploid varieties | | | | |
| Y-1 | 1994 | Sirsa | 16.33 | 0.061 |
| Y-1 | 1994 | New Delhi | 18.21(H) | 0.054(L) |
| Y-1 | 1995 | Coimbatore | 15.85(L) | 0.063(H) |
| Range within variety (H-L) | | | 2.36 | 0.009 |
| Average value | | | 16.79 | 0.059 |
| Standard Deviation | | | 3.12 | 0.008 |
| AKA-5 | 1994 | Sirsa | 15,21(L) | 0.065(H) |
| AKA-5 | 1995 | New Delhi | 16.79(H) | 0.059(L) |
| AKA-5 | 1995 | Coimbatore | 16.39 | 0.061 |
| Range within variety (H-L) | | | 1.58 | 0.006 |
| Average value | | | 16.13 | 0.062 |
| Standard Deviation | | | 0.82 | 0.003 |
| SRT-1G. Cot.10 | 1992 | Nagpur | 25.38(H) | 0.039(L) |
| SRT-1G. Cot.10 | 1992 | Coimbatore | 23.70 | 0.042 |
| SRT-1G. Cot.10 | 1994 | New Delhi | 20.13(L) | 0.049(H) |
| Range within variety | | | 5.25 | 0.010 |
| Average value | | | 23.07 | 0.043 |
| Standard Deviation | | | 2.68 | 0.005 |
| AKH-4 | 1995 | New Delhi | 21.80(H) | 0.045(L) |
| AKH-4 | 1995 | Nagpur | 19.96 | 0.050(H) |
| Range within variety (H-L) | | | 1.84 | 0.005 |
| Average value | | | 20.88 | 0.047 |
| Standard Deviation | | | 1.30 | 0.003 |
| Maljari | 1992 | Nagpur | 14.19 | 0.070 |
| Maljari | 1992 | Coimbatore | 13.18(L) | 0.075(H) |
| Maljari | 1994 | Sirsa | 17.68(H) | 0.056(L) |
| Maljari | 1995 | New Delhi | 16.81 | 0.059 |
| Range within variety (H-L) | | | 4.50 | 0.019 |
| Average value | | | 15.46 | 0.064 |
| Standard Deviation | | | 2.12 | 0.009 |

H : Highest value ; L : Lowest value