Inheritance study for lint colour in naturally brown coloured upland cotton (Gossypium hirsutum L.)

R. Nivedha1*, S. Rajeswari2, N. Premalatha2 and N. Sritharan3

1Centre for Plant Breeding and Genetics,
2Department of Cotton, Centre for Plant Breeding and Genetics,
3Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore – 03, Tamil Nadu, India.
*E-Mail: nivedharakkimuthu@gmail.com

Abstract
The naturally coloured cotton is the age old crop holding evidence in ancient civilization. Over the course of time, the machine spinnable superior white linted varieties replaced the cultivation of coloured cotton which having poor yield and fibre quality. In the present era, concerns about pollution and toxicity caused by synthetic dyes and dyeing industries have resurrected the interest of naturally coloured cotton. In view of eco-friendly processing, improving the fibre characteristics of naturally coloured cotton is the need of the hour. The study of genetics behind the colour development serve in developing coloured linted varieties with enhanced fibre quality by crossing with superior white linted lines. The present study involved studying F2 populations of direct and reciprocal crosses between two naturally coloured (Parbani American and Red 5-7) and one white fibred (MCU 5) upland cotton. The F1 was intermediate to parents in fibre colour. The phenotypic ratio in F2 was observed to be 1:2:1 proving the inheritance to be governed by single incompletely dominant gene. But a lot of variation for colour intensity in F2 could be related to more than one loci or the presence of modifiers. This could be further examined at molecular level in future.

Keywords
Naturally coloured cotton, inheritance, brown fibre

INTRODUCTION
Cotton, the king of fibres, is the most fundamental raw material for textile industries worldwide. The cotton fibre with accumulated pigments in lumen is called naturally coloured cotton. Veerland James (1999) reported that naturally coloured cotton dates back to more than 5000 years. All the four cultivated species and about 22 wild species of cotton possesses coloured lint (Kranthi, 2014). In India, brown linted tree cotton (G.arboreum) viz., Coconada1 and Coconada 2 were under commercial cultivation in Andhra Pradesh and exported to Japan (Murthy, 2001). The lint exists in various hues and the most common among them are brown and green colour. The brown colour intum has many shades ranging from dark brown to mahogany red. Depending on the intensity it is named as light brown, khaki/camel colour, brown, dark brown/chocolate colour, dirty grey, tan and red (Singh et al., 2001). The naturally coloured cotton eliminates the process of dyeing in fabrics. This results in curtailment of harmful discharge from dyeing industries into water bodies risking human health and environment. About one half of the cost of preparing textiles can be reduced by eliminating dyeing process (Pooja and Anita., 2018). Furthermore, the fabrics made from naturally coloured cotton are steadfast that does not undergo quick fading compared to synthetically dyed clothes. Despite the economic and ecological benefits, commercial cultivation of naturally coloured cotton is very limited by the farmers because of few drawbacks. The most important among them is the low quality fibres. The fibre colour is negatively correlated with fibre yield and quality (Feng et al., 2015). In addition to low quality, the limited range of colours, instability of lint colour on over exposure to sunlight, need for isolated field to avoid contamination of white cotton and lack of standard criteria for quality classification have reckoned
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for its limited cultivation (Matusiak and Frydrych, 2014). The development of fibre colour in cotton is genetically controlled. The brown colour was reported to have dominant heredity (Wang et al., 2012). Kohel (1985) studied the genes for fibre colour and reported six loci for brown colour viz., Lc1 and Lc2 (medium brown), Lc3 (dark brown), Lc4, Lc5 and Lc6 (light brown). The inheritance study of fibre colour will enable us to determine the gene action involved in lint colour which could be utilized in developing coloured varieties by crossing white linted with colour linted lines.

MATERIALS AND METHODS

The study material comprised of two varieties of naturally brown coloured cotton viz., Parbani American (medium brown) and Red 5-7 (dark brown) and one white linted cotton variety MCU 5 obtained from the Department of Cotton, Tamil Nadu Agricultural University. Both direct and reciprocal crosses were made in such a manner that the coloured varieties and white variety served as parents in either directions resulting in four cross combinations (Table 1). The F₁ hybrids observed to possess brown coloured lint intermediate to both parents were forwarded to F₂. The segregating F₂ generations were studied in order to assess the inheritance pattern of lint colour.

The experiment was conducted during kharif 2019 at field no 4C at the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore. Since cotton is an often cross pollinated crop, in order to avoid contamination of white cotton, the field was raised under isolation. Each cross was raised in 20 rows with the spacing of 90 x 45cm. The agronomical and plant protection measures were carried out from sowing to harvest as per the standard recommendation. In segregating population, selfing was carried out to forward the selected lines to next generation. Each and every plant of all the crosses were selfed using wet clay method and thread method. Selfing was done continuously for 20 days from 70 days after sowing.

Table 1. Details of cross combinations made with number of plants studied in each cross

| S No | Crosses                     | Number of plants studied in F₂ |
|------|-----------------------------|-------------------------------|
| 1    | Parbani American x MCU5    | 209                           |
| 2    | MCU5 x Parbani American    | 221                           |
| 3    | Red 5-7 x MCU5             | 212                           |
| 4    | MCU5 x Red 5-7             | 204                           |

| P      | Parbani American | MCU 5         |
|--------|------------------|---------------|
| X      | RHS01-167 B      | White         |
| F₁     | Intermediate     |               |
| F₂     | segregants       |               |
| RHS01-167 A | RHS01-167 B | RHS01-167 C | RHS01-167 D | RHS01-N167 B |
| RHS01-N167 C | RHS01-168 D | RHS01-159 A | White        |

Fig. 1. Pictorial representation of Parbani American x MCU 5 with colour grade matched with The Royal Horticultural Society colour chart.
The $F_2$ populations of four crosses under study were visually inspected and scored for lint colour. The brown colour had segregated into many shades ranging from light to dark brown (Figure 1, 2, 3 and 4). The colour grades for different shades were given by matching with the universally accepted colour chart of The Royal Horticultural Society (2001).

Fig. 2. Pictorial representation of MCU 5 x Parbani American with colour grade matched with The Royal Horticultural Society colour chart

Fig. 3. Pictorial representation of Red 5-7 x MCU 5 with colour grade matched with The Royal Horticultural Society colour chart
Chi square test was used to assess the Mendelian inheritance pattern for lint colour in \( F_2 \) segregating population. The significance of difference between observed and expected frequencies or ratios was studied using the method given by Klug and Cummings (1999).

\[
\chi^2 = \sum \frac{(O-E)^2}{E}
\]

Where,

- O = Observed frequencies
- E = Expected frequencies

The chi square values tested against table values with \( n-1 \) degrees of freedom (\( n \) is the number of classes) or the probability value were taken into consideration for rejection or acceptance of Null hypothesis.

### RESULTS AND DISCUSSION

In both direct and reciprocal crosses, the fibre colour of each plant in \( F_1 \) was intermediate to that of the parents. This confirmed that the brown colour of fibre had nuclear inheritance and exhibits incomplete dominance or partial dominance over white (Ware, 1932; Richmond, 1943; Xianlong, 2004). On visual inspection of individual plants in \( F_2 \), the intensity of brown colour varied showing eight different shades under two groups (Greyed orange – 167, N167, 168 and Orange white – 159) in colour chart of The Royal Horticultural society. Since discrete grouping of colour was not possible, the plants having similar and slightly darker shades than brown parent were grouped under brown and those with lighter shades than brown parent were taken under intermediate category.

### Table 2. Chi square test for the cross Parbani American x MCU 5

| Phenotype | Class | Observed | Expected | \( \chi^2 \) value | p value |
|-----------|-------|----------|----------|--------------------|---------|
| Brown     | 1     | 41       | 52.25    | 3.49               | 0.17    |
| Intermediate | 2  | 115      | 104.5    |                    |         |
| White     | 1     | 53       | 52.25    |                    |         |
| Total     | 4     | 209      | 209      |                    |         |

For interpretation of results, the chi square test of all the crosses indicated no significant deviation from the inheritance pattern of 1:2:1 phenotypic ratio. In the first cross Parbani American x MCU5, among 209 \( F_2 \) plants, 41 plants possessed brown fibre, 115 plants had intermediate phenotype and 53 plants were found to have white fibre (Table 2). In the corresponding reciprocal cross (MCU5 x Parbani American), among 221 plants studied, 47 carried brown coloured fibre, 123 intermediate types and 51 plants had white fibre colour (Table 3). The segregating population of third cross (Red 5-7 x MCU5) consisted of 43 brown linted plants, 122 intermediate coloured and 47...
plants of white fibre among 212 observed plants (Table 4). The reciprocal cross (MCU5 x Red 5-7) was observed to possess 43 brown linted plants, 116 intermediate phenotype and 45 white linted plants in a population of 204 F₂ plants (Table 5). In the chi square analysis of all the crosses, the p value is more than 0.05 which implied the acceptance of null hypothesis. This indicated that all the crosses fit 1:2:1 expected ratio at a significant level of α=0.05. It provided sufficient proof for the goodness of fit for monohybrid inheritance pattern portraying that brown colour of naturally coloured cotton was controlled by single incompletely dominant gene. Similar results were obtained by Shaohua et al (2008) and Hinchliffe et al (2016).

Table 3. Chi square test for the cross MCU 5 x Parbani American

| Phenotype | Class | Observed | Expected | χ² value | p value |
|-----------|-------|----------|----------|----------|---------|
| Brown     | 1     | 47       | 55.25    | 2.97     | 0.23    |
| Intermediate | 2 | 123      | 110.5    |          |         |
| White     | 1     | 51       | 55.25    |          |         |
| Total     | 4     | 221      | 221      |          |         |

Table 4. Chi square test for the cross Red 5-7 x MCU 5

| Phenotype | Class | Observed | Expected | χ² value | p value |
|-----------|-------|----------|----------|----------|---------|
| Brown     | 1     | 43       | 53       | 4.98     | 0.08    |
| Intermediate | 2 | 122      | 106      |          |         |
| White     | 1     | 47       | 53       |          |         |
| Total     | 4     | 212      | 212      |          |         |

Table 5. Chi square test for the cross MCU 5 x Red 5-7

| Phenotype | Class | Observed | Expected | χ² value | p value |
|-----------|-------|----------|----------|----------|---------|
| Brown     | 1     | 43       | 51       | 3.88     | 0.14    |
| Intermediate | 2 | 116      | 102      |          |         |
| White     | 1     | 45       | 51       |          |         |
| Total     | 4     | 204      | 204      |          |         |

Eventhough the ratio supported single gene inheritance, the varying intensity of brown colour in F₂ rather than discrete two phenotypes paved way for the possibility of more than one locus responsible for colour as proposed by Kohel (1985). Wang et al (2014) reported that the modification of minor genes could have accounted for variations in F₂ population. Therefore, further study in F₃ generation and molecular confirmation of homozygous and heterozygous plants will aid to judge the number of loci governing lint colour trait. Nevertheless, the present study throws light on the potential for bringing colour in the present white linted lines having high fibre quality by exploiting the knowledge of incomplete dominant heredity pattern of brown colour in future breeding programmes.

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