CHAPTER 3
MATERIALS AND METHODS

The seeds of promising strain NA-39 (diploid) G.arboreum were exposed to physical and chemical mutagens.

3.1.1 PHYSICAL MUTAGENE TREATMENTS:

For each treatment 500 bold seeds of promising strain NA-39 were exposed to irradiation for 15KR, 20 KR and 30 KR dose at the rate of 7KR/min. using 27 Co\textsuperscript{60} source at Division of Genetics. Indian Agricultural Research Institute, Pusa, New Delhi -12. Treated seeds were sown immediately by dibbling one seed per hill at a distance of 22.5cm between plants and 45cm between rows. Similarly equal number of untreated seeds of NA-39 were sown as a control.

3.1.2 CHEMICAL MUTAGENES TREATMENT: Ethyl Methyl Sulphonate (EMS) (Estaman Chemicals) was used as a chemical mutagen. Fully developed 500 seeds of NA-39 (For each treatment) were soaked in freshly prepared EMS solution for 24 hours with different concentrations 0.1%, 0.2%, 0.3%, 0.4% and 0.5% on mechanical shaker at room temperature with continuous shaking. EMS treated seeds of different treatments then washed thoroughly with running tap water and immediately sown. Equal quantity of presoaked (500 seeds soaked for 24 hrs. in water) seeds of NA-39 were sown as a control as above.
To raise M2 generation in the form of M2 families, the seeds of M1 individual plants were collected separately. The group of individuals derived from the seeds of a single M1 plant contributed M2 family (single plant seeds sown in single row). Each plant at flowering stage was selfed to avoid cross pollination. M2 families of each treatment including control were used to raise polygenic trial to study polygenic mutations. Each M2 family was represented by a single row, populations were screened for both macro and micromutation. The rest of the families of each treatment were raised in unreplicated single row progeny. Two rows of respective control plant progenies were grown, there families were screened for visible mutation only. Point mutation was observed in two plants of 15 KR gamma-Ray treatment. Only one branch was fertile and remaining whole plant was unfertile with out flowers/bearings.

Observations on morphological changes and special features were noted and tagged, individual plant for leaf size, flower colour, pollen sterility, seed size, seed index, seeds per locule, naked seeds, boll size, boll bearing, change in plant architecture, like dwarf plant, branching, clusters per plant, pods per plant. Early maturity, yield per plant, fibre staple and fineness, ginning percentage, etc. Individual plants with noted specific characters were harvested separately, to grow further generations and to study the performance. Field experimental observations (on
germination, sterility. Chlorophyll mutations and mortality) though studied in detail are not included in the present investigation because main emphasis of this experiment was to study the effect of mutagens on fibre properties.

Superior plants for quantitative and qualitative characters or gross morphological changes were selected and harvested separately for further testing. Seeds obtained from screened plants were sown in plant to row progeny with three replications. Phenotypic variations for qualitative characters were evident. Mutants showing superiority over control for boll bearing, better quality parameters were selected in M3 generation.

Progenies of the selected plants were sown in a plant to row progeny to advance the generation, after throughout screening from large plant population. Finally ninety one derivatives were tested on large scale in RBD with three replications. Border row around the experimental field was sown to avoid border effects. Obtained derivatives showed less variations in morphological characters but possessed wider range of variability for qualitative and quantitative characters. Further selection and progeny testing of selected materials was carried upto M5 generation. Derivatives of M5 generations were screened by adopting parameters like mean, range, coefficient of variation, genetic gain and correlations. Coefficient of variation and genetic advance in the population of each treatment were estimated by statistical parameter. Genotypic co-rrelation,
helped in understanding the efficacy of each treatment in breaking the undesirable linkages and raising intensity of desirable association. The successful performance of mutant line depends on beneficial mutation and the genotype of which agronomic attributes such as adaptibility, resistance, quality and yield, are studied.

In each generation, seeds were planted with inter and intra spacing of 45 cm and 22.5 cm along with boarder rows. Recommended fertilizer dose of 50:25:25 NPK Kg/ha was applied. Plant protection measures for protecting crop from bollworm were adopted, as and when required. During the experimental study from M2 to M5 generation seasons were normal with average rainfall around 900 mm.

3.2 CHARACTERS STUDIED FROM M2 TO M5 GENERATION:
Detailed fibre quality studies were carried out at quality evaluation and improvement unit of central institute for research on cotton technology. Cotton Research Station Nanded. Standared methods (physical fibre properties and microscopic characters) of fibre tests (were adopted as per guidelines given in 'Hand Book of Methods of Tests by Dr. V. Sundaram. Methods of tests carried out are briefly described.

3.2.1 MEAN FIBRE LENGTH (MFL) occupies dominant position as it influences, to a large extent the spinnability of cotton and it is of great importance in commercial point of view as price of cotton mostly depends on this property of fibre. Mean fibre length is the arithmatic mean of the length of all
the fibres present in the small but representative cotton sample, is determined by Bolls sorter instrument. This method is based on mechanically sorting of fibres from silver in order of length. The shortest fibres being deposited first and longest last of all. The fibres in each length group are measured on sensitive Torsion balance as per weight-length distribution. Mean fibre length is calculated as given formula: \( L = \frac{WL}{W} \)

3.2.2 FINENESS: Fibre fineness is a measure of size, diameter, linear density or mass per unit length expressed as linear density in micrograms/inch or in millitex. Fineness denotes the size of cross sectional dimensions of the fibres which is irregular. Hence direct measurement of area of cross section is difficult and laborious. Indirect method is used for measurement of fibre fineness i.e. resistance to the flow of air by fibre plug depends on the specific surface area (surface area per gm) of fibre. Micronaire instrument is used for determination of fineness i.e. the weight of one inch single fibre in micrograms. This micronaire value is a measure of air permeability of mass of cotton fibre under specified condition. The air at definite pressure i.e. (1.75 kg/cm²) is forced through 3.24 grams (50 grains) of lint sample kept in cylindrical chamber and volume rate of air flow is measured as a micronair value.

3.2.3 MATURITY COEFFICIENT is percentage of mature, half mature and immature fibres.

\[ M_c = \frac{(M + 0.6H + 0.4I)}{100} \]
Fig. 5
MATURE

Fibre maturity.

HALF-MATURE

IMMATURE

Fig. 6
Fibre crosssections.
coefficient is determined by use of 18% caustic soda solution to irrigate the fibres and examined under microscope to classify fibres in three categories as mature, half mature (= 0.6 mature fibre) and immature fibre which is equivalent to 0.4 mature fibre.

\[
\text{Mature fibre percentage (M\%)} = \frac{\text{No. of Mature fibres}}{\text{Total No. of fibres}} \times 100
\]

There is another indirect method for determination of Mc by spacer technique used in micronaire instrument. It has been noted that 'the resistance offered to the flow of air by cotton plug is dependent not only on the fineness but also on maturity of fibres' by Hertel and Craven, who designed the Arealometer for measuring the fineness and maturity of cotton. They showed the difference in specific surface area of a cotton sample determined at two degrees of compression in the Arealometer was related to maturity of the sample. It is observed that the difference in micronaire values of various samples measured with 3/8 inch spacer and without the spacer were highly correlated with the maturity coefficient (Mc). A regression equation connecting these two factors has been worked out.

\[
\text{Maturity coefficient (Mc)} = Y = 0.1753x + 0.3934
\]

where \( x = (\text{WS} - \text{NS}) \) i.e. difference between micronaire readings observed with spacer and (without) no spacer.

Estimation of fibre maturity coefficient by using 3/8 inch spacer. The plunger compressing the fibre is raised and lint sample is compressed to a lesser extent than without
spacer, i.e. degree of compression is altered. Due to resiliency of fibres different micronaire value is obtained and fibre Maturity coefficient (Mc) is estimated.

3.2.4 FIBRE STRENGTH : The maximum load which a material can take, when stretched in one direction before it breaks is known as tensile strength.

Breaking Strength : The maximum tension the fibre is able to sustain before it breaks. It depends on inherent places and area of cross section of the fibre. Intrinsic Strength is calculated by dividing the breaking load by fibre weight per unit length known as fibre tenacity. It is related to spinning quality.

Stelometer instrument used for measuring fibre bundle strength and elongation, which is known as strength elongation meter. This is a pendulum type instrument working on the principal of constant rate of loading. (i.e. 1 kg/sec) at 27°C and 65% relative humidity (R.H). Tensile strength of cotton fibre is influenced by time taken to rupture the specimen, the test length of specimen and relative humidity in which sample is conditioned and tested. A lower ‘Time to break’ results in a high breaking load. ‘Time to break’ can be controlled by ‘rate of loading’, ‘rate of traverse’ or ‘rate of extension’. Fibre strength is influenced by ‘gauge length’ used. The lower strength is obtained for longer gauge length because presence of weak places appearing in the test length. Lord Pointed out that the strength of fibre is the strength of weak place, no
matter how much stronger the specimen is at other places. Fibre strength at '0' gauge length do not give the information of weak places in the fibre as they do not come in to play. 1/8 gauge strength (T1) Bundle Strength measured at 1/8 inch (3.2mm) gauge length has better correlation with yarn strength.

\[
\text{Stelometer value at "0" gauge length (g/tex) } = \frac{\text{Breaking strength of bundle in Kg} \times 11.81}{\text{weight of bundle in mg.}}
\]

'1/8' inch gauge (g/tex) = \frac{\text{Breaking strength in kg} \times 15}{\text{weight of bundle in mg.}}

'1/4' inch gauge (g/tex) = \frac{\text{Breaking strength in kg} \times 18.2}{\text{weight of bundle in mg.}}

For determination of the breaking strength in g/tex on stelometer. When zero gauge test length is used, this length would be 11.81 mm which is the combined width of two clamps.

While for '1/8' gauge length, the test specimen width including spacer and clamps becomes 15mm and for '1/4' gauge this length would be 18.2mm, which is the combine width of clamps and two spacers.

3.2.5 ELONGATION: The elongation indication on stelometer gives percentage elongation (E1) at '1/8' or 3.2 mm) gauge length. This observed reading should be multiplied by 0.8 to compensate for slipage of fibres in the clamp. Same method is used for elongation E2 at '1/4' inch (6.4 mm) gauge length. Strength depends on gauge length which
influences both strength and elongation. A '0' gauge length, chiefly the fibrillar orientation determine the strength, at higher gauge length in addition to orientation structural reversal and crimp play important role in determining the strength. Pierce Theory, 'weakest link' the decrease in strength is closely proportional to log of gauge length increase.

3.2.6 TOUGHNESS: Toughness or work of rupture is obtained by;
= 1/2 Bundle tenacity at 3mm gauge X fractional elongation at break at 3mm
= 1/2 T1xE1 (i.e. half the product of bundle strength and elongation at 3mm)

Fibre bundle strength at 3mm gauge length

3.2.7 STIFFNESS = -----------------------------------------
Elongation % at 3mm

(Toughness showed good association with orientation where as stiffness has no association)

<table>
<thead>
<tr>
<th>Strength Uniformity</th>
<th>T1</th>
<th>S2</th>
<th>Stelo value at '1/8'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>T0</td>
<td>S1</td>
<td>Stelo value at '0'</td>
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</table>

<table>
<thead>
<tr>
<th>Strength Uniformity</th>
<th>T2</th>
<th>S3</th>
<th>Stelo value at '1/4'</th>
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<tbody>
<tr>
<td>Ratio 2</td>
<td>T0</td>
<td>S1</td>
<td>Stelo value at '0'</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Elongation Uniformity</th>
<th>E1</th>
<th>% Elongation at break '1/8' gauge</th>
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<tbody>
<tr>
<td>Ratio</td>
<td>E2</td>
<td>% Elongation at break '1/4' gauge</td>
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Mechanical properties are governed by the molecular and super molecular structure of the fibre.

3.3 MICROSCOPIC STUDIES OF FIBRE STRUCTURE:

The cotton fibre is the epidermal out growth of the
Microfibrillar morphology of cotton fibre.

FIG. 1 Schematic Diagram of cotton fibre
seed. It is a single biological cell which is the purest form of naturally occurring cellulose. Morphological fine structural studies of cotton fibre revealed that cuticle is the outermost layer consisting of pectin, fats and waxes embedded in a criss-cross network of the primary wall (0.2u thick) constituted by microfibrils inclined at 70° to the fibre axis.

Secondary wall constitutes major part of the fibre i.e. 90% of cellulose is deposited in successive growth layers (5u thick) and is made up of lamellae, closely packed microfibrils. The secondary wall is divided in three layers. The first layer S1 is the transition layer between the primary wall and the S2 layer, consists of fibril bundles inclined about 45° to 70° to the fibre axis.

The S2 layer consisting of several sheets of parallel microfibrils inclined at 20°-30° to the fibre axis constitutes major part of secondary wall. These bunch of parallel microfibrils reverse the direction of spirality at several points along the length of the fibre, they are reversals.

The S3 layer of secondary wall lies with lumen, it is very thin and not readily visible.

Lumen is the innermost part of the fibre, it is not visible in fully matured fibres but it can be seen clearly in immature fibres by swelling. Void lumen in immature fibres creates difficulties in spinning and dying due to weak fibre with less cellulose deposition.
Fibres emerging from seed coat grow mainly in length. Initially fibre is hollow, cylindrical, afterwards secondary cell wall development starts, cellulose is deposited inwardly in successive lamellae or growth layers till ball bursts. The turgid living cells exposed to the sun and collapse into flattened tubes due to desiccation and twist along the length of fibre. These are convolutions. Hence the cross-section of dried cotton fibre is irregular (doughnut shaped).

3.3.1 **CONVOLUTIONS** :

Cotton fibre dried after boll opening looses its tubular shape and becomes an irregularly flattened tube twisted through $180^\circ$ in S to Z direction along the length. These twists are convolutions. Balls observed that convolutions in dry cotton fibre are due to underlying spiral structure of cellulose. Meredith reported that the number of convolutions per unit length and convolution angle of fibre depends on the variety (varietal character), convolutions vary from 30 to 75 per cm for different varieties. Finer cottons have more convolutions per unit length as compared to coarser fibres. Immature fibres have fewer convolutions than mature fibres, i.e. thin walled fibres as well as compared to thick walled fibres show very few convolution/cm.

Convolutions occur all along the length of fibre except near the tip. Convolutions in the fibre contribute to the
frictional behaviour. The convolution angle influences the fibre strength, large convolution angle generally associated with low strength.

3.3.1.1 NUMBER OF CONVOLUTIONS: Ten fibres are mounted on a slide and a drop of liquid paraffin is added placing a cover slip over them to examine the fibres under microscope at 250X magnification. Only central 5mm region under cover slip is observed and the number of convolutions are counted. Average number of convolutions per mm of fibre is expressed.

3.3.1.2 CONVOLUTION ANGLE: Convolutions follow same direction as underlying fibrils. As fibrils reverse in the direction of spirallation, the convolutions also change the direction accordingly, so that increase in spiral angle correlates with increase in convolution angle.

Average convolution angle is estimated by measuring the pitch of convolution (c) and ribbon width(R) -

\[ \tan \theta = \frac{\pi}{2} \left( \frac{R}{c} \right) \]

II

\[ R \tan \theta = \frac{\pi R}{2} \left( \frac{R}{c} \right) \]

Ribbon width and pitch of convolution are measured by compound microscope, at least ten observations are required for average convolution angle. Ribbon like fibres, show a tendency to curl at the edges the measured ribbon width is generally less than its real value. The corrected average ribbon width is given by -
\[ R = \frac{\sqrt{\pi w}}{\varphi S} \left[ 1 - \frac{2}{\pi} \right] \left[ 1 - \sqrt{1-S} \right] \]

\( w \) - the fibre weight per unit length
\( \varphi \) - the density of the fibre
\( S \) - degree of thickening, defined as the ratio of the area of cross section of the cell wall to area of the circle with the same perimeter

For Indian cottons degree of thickening = 0.00294 \((M-I) 0.409\)

where \( M \) & \( I \) are the percentages of mature and immature fibres in a sample. Meredith observed good correlation between convolution angle and pressley strength index \( r = -0.87 \), Dependence of X-ray angle on convolution angle \( r1 = 0.84 \).

3.3.2 STRUCTURAL REVERSALS:

Secondary wall consists of layers or lamellae of cellulosic micro fibrils oriented in a spiral along the axis. The fibrilar strands in every layer change their direction of spiral from \( S \) to \( Z \) or \( Z \) to \( S \) very frequently along the length of fibre. The direction of fibrilar orientation in consecutive layer are opposed to each other but reverse their direction at the same position through out the section of secondary wall. The region at which the spiralling fibrils change their direction of spiral are structural reversals. This is an unique feature of the cotton fibre and appear as a dark region when viewed under crossed polarids. At this region fibre possess a high lateral and longitudinal order.
Molecular structure of cellulose (fibre).

Fig. 3
Reversals in cotton fibre.

Fig. 4
convolution angle of fibre.
Betrabet et al observed that the average number of reversals in cotton fibre is a genetic character and varies from 2 to 27 per cm. Coarser cotton showed less number of reversals than finer cottons.

3.3.2.1 Measurement of Number of Reversals/cm. : Fibres are mounted on glass slide stright and parallel to each other and viewed under microscope between polariser and analyser which are placed with their polarisation direction at right angles to each other so that view is dark when the fibre is absent. The slide is positioned such that the fibres are either parallel or perpendicular to the polarisation direction. The fibre appears illuminated except at the reversals where it is dark.

Standard method for measurement of reversals per cm is adopted. Fibres are mounted straight and parallel on glass slide and irrigated with a drop of 18% NaOH. (Sodium Hydroxide) of mercerising strength to remove convolutions and surface striations. The swollen fibres are then examined between crossed polaroids. Coverslip of 10 mm is used to count reversals per cm. 3.3.3 Fibre Structural Studies :

X-rays are electromagnetic waves having short wave length (\( = 1 \, \text{Å}^{-1} \)). X-ray diffraction is used to measure the angle of orientation of cellulose fibrils inside cotton fibres. It is also used to measure the crystallinity of the fibre.

When an X-ray beam is incident on a single crystal the rays get diffracted from various crystal planes in addition to the primary. The position of the diffraction pattern
Schematic Diagram of Philips Model 1130 x-ray generator with diffractometer attachment

1. current regulator; 2. transformer;
3. rectifier; 4. stabiliser;
5. x-ray tube; 6. powder sample;
7. slit system; 8. filter;
11. pre-amplifier; 12. line amplifier;
13. pulse height analyser; 14. timer;
15. scaler; 16. rate meter;
17. recorder.
generated by scattering from crystal planes governed is
Bragg's law

\[ 2d \sin \theta = n\lambda \]

Where \( d \) is the distance between successive similar
planes in the crystal

\( \theta \) is the angle of incidence

\( \lambda \) is the wave length of X-radiation

\( n \) is the order of the diffraction maxima.

If the material is in the form of a powder, it contains
crystals oriented randomly at all possible angles so that
there will be some crystal faces at the appropriate angles
for diffraction. The X-rays diffracted by a given set of
planes form a cone. The axis of the cone is the primary X-
ray beam and the space angle at the vertex is equal to 20.
If the sample is a bundle of fibres arranged parallelly, the
rings are arcs on the equator and meridian. The arcs on
the equator are due to the planes parallel to the fibre axis
and those on the meridian originate from planes perpendicular
to the fibre axis.

The breadth of diffraction arcs is decided by the size
of crystallites. The length of the arcs is decided by extent
of deviation from parallelism. **Experimental Setup** - fig,
gives a block diagram of an X-ray generator. A highly
stabilized 10-80 KV DC source is fed to a sealed X-ray tube.
The tube has four output windows. Two of the window give
line focus and the remaining two point focus. The target is
Cu (Copper). The radiation employed for the present study is CuKα = 1.5482 Å. Philips wide angle X-ray diffractometer attachment is used in conjunction with the above generator, to record the intensity of diffraction as a function of diffraction angle.

Reflection geometry is employed for this purpose. The diffractometer is aligned with the X-ray beam in line focus. The radial intensity can be measured in the range 0° to 40°. It can be either scanned for this entire range or measured at any preset angle. The detector used is a Geiger Mullar counter.

3.3.3.1 CRYSTALLITE ORIENTATION:

This describes the inclination of cellulose molecules to the fibre axis and governs the tensile properties of the fibre. Crystallite orientation distribution refers to the distribution of small crystallites of the ordered aggregates of cellulose molecule with respect to cotton fibre axis.

Spiral angle describes the angle made by the fibrils containing the crystallites about the fibre axis in natural fibres. These angles do not have fixed values but differ at different places along the fibre and also in various growth layers. But variation is within a narrow range.

The beam of X-rays when passed through a single fibre or bundle of fibres produce a diffraction pattern. The length of arc measures the extent of variation in orientation. If it is small the variation in orientation is small or the extent of orientation is high. The degree of orientation is
expressed in terms of Herman's orientation factor. Generally 40% to 50% X-ray angle, which actually gives the angle at which the diffracted X-ray intensity drops to 40% or 50% of the maximum diffracted X-ray intensity.

The Herman orientation factor is defined as:

$$f_x = 1 - \frac{3}{\sin^2 d}$$

where $$\sin^2 d$$ is the expectation value of $$\sin^2 d$$ and is measured from the crystallite orientation distribution curve or profile by measuring intensity correspond to each azimuthal angle and computing $$\sin^2 d$$ as given below;

$$\sin^2 d = \frac{\int I(d) \sin^2 d \cos d \, d}{\int I(d) \cos d \, d}$$

where d is angle measured from position of maximum intensity. Increase in crystallite orientation may improve tensile strength, stiffness etc. [Fibre crystallinity has important bearing on the properties of the fibre]

3.3.4 CRYSTALLINITY :-

Crystallinity is known to influence the physical and chemical behaviour of cotton fibres. Infrared spectroscopy is also used to measure crystallinity. Crystallinity is found to have co-relation with fibre properties like tenacity, extensibility and fineness.

Methods of measuring crystallinity are both destructive and non-destructive type. An attacking chemical penetrates the disordered regions of the polymer at a faster rate than in the crystalline region. Hydrochloric acid is an ideal
chemical for attacking, destroying and then dissolving away the broken residues of the disordered regions of cellulose polymer. It causes the molecules in these areas to split into small fragments and there after dissolution. The unreacted part (measured by molecular weight of the residue polymer) is the amount of crystalline polymer.

X-ray methods are most widely used as non-destructive methods. But this method works better if the crystallinity is higher and size of the crystals are large at least > 500 Å. The method does not give satisfactory results if imperfections are more.

3.3.4 INFRARED SPECTROPHOTOMETRY: Another important method of finding the ratio of crystalline to amorphous material is by infrared spectrometry. A molecule is a group of atoms bound together by elastic bonds formed by the electron clouds around the atomic nuclei. These elastic bonds allow the atoms to vibrate about a mean position. The frequency of vibration of an atom is determined by its mass and the strength of the bond between them. Each chemical bond, C-H, C-C, O-H, C-O, etc., has a characteristic frequency. Which lie in the range $1.2 \times 10^3$ to $0.5 \times 10^{13}$ cycles per second. When a continuous spectrum of infrared rays falls on matter the vibrating molecule interacts with the incident energy resulting in resonant absorption. In a complex molecule made up of N atoms there are in general $3N-6$ possible fundamental frequencies of vibration. Only those
vibrational modes which are accompanied by a change in the electric dipole moment of the molecule as a whole are infrared active and hence the actual number of absorption bands will be considerably less. Nevertheless, overtones of the fundamental frequency can appear in the spectrum, though with progressively reduced intensities. The infrared absorption bands of cellulose and other similar polymers lie in the frequency range 4000 cm\(^{-1}\) to 200 cm\(^{-1}\).

The characteristic absorption frequencies show variations depending upon the environment of the group of atoms and the consequent inductive effects. A study of precise vibrational frequencies could give valuable information about the configuration as well as the structure of molecular aggregates. The intensity of an absorption band is related to the number of absorbing groups or to the concentration of the group. Infrared method could be employed for a variety of quantitative analysis. Infrared spectroscopy is used to measure relative orientation of different bonds leading to the determination of molecular conformations. The degree of molecular orientation in fibres can be assessed if molecular configuration or conformation are fully known.

In cellulose material OH groups are hydrogen bonded in crystalline regions. Hence if the material is crystalline the Hydrogen in the crystalline part cannot be replaced by Deuterium. The amount of H replaced by Deuterium is the measure of amorphous part. Since OH bond vibrations and OD
vibrations are at different Infrared frequencies from their intensities crystallinity can be measured. OH stretching frequency at 3360 cm$^{-1}$ and OD at 2530 cm$^{-1}$ are used to characterize crystallinity of cotton by Mann and Marrinan. Deuteriation is carried out on the substance in the form of film.

O’connor et al used 1429 cm$^{-1}$ bond and 893 cm$^{-1}$ band, for characterization of cellulos. 1429 cm$^{-1}$ band is due to the bending deformation of CH$_2$. When crystallinity is reduced intensity of 1429 cm$^{-1}$ band decreases whereas that of 893 cm$^{-1}$ band increases. Intensity changes of these bands were observed during mercerization also. 1372 cm$^{-1}$ band increases in intensity on crystallization. This band had the advantage of being observed for both cellulose I and II. It was compared with the band at 2900 cm$^{-1}$ which was used as internal standard.

**CHOICE OF BANDS** - Mann & Marriran defined crystallinity as the fraction of OH group which are hydrogen banded in a regular crystalline manner and remain accessible to deuterium exchange. They calculated the absorption at 3360 cm$^{-1}$ due to OH and 2350 cm$^{-1}$ due to OD stretching and used the infrared ratio as a measure of crystallinity. O’connar et al used the band at 1429 cm$^{-1}$ and 893 cm$^{-1}$ to measure crystallinity. The band at 1429 cm$^{-1}$ is due to CH$_2$ bending. The band at 893 cm$^{-1}$ is due to the vibration of C1 and the four atoms attached to it. The ratio of absorbance at 1429 cm$^{-1}$ and 893 cm$^{-1}$ showed a pronounced increase as crystallinity of the
sample is reduced.

Nelson & O’connor used the band at 1372 cm\(^{-1}\) for crystallinity determination. They found the band to be intense in both cellulose I and cellulose II and the intensity was found to decrease on decrystallization.

Iyer et al used intensity of band at 1372 cm\(^{-1}\) & 342 cm\(^{-1}\) as measure of cellulose crystallinity. They have observed that crystallinity calculated from intensity of 342 cm\(^{-1}\) agrees better with X-ray results.

Blackwell studies low infrared and Raman spectra of cellulose, Michel has studied infrared spectra below 600 cm\(^{-1}\) but they have not identified the band at 342 cm\(^{-1}\). However Roy who has carried out theoretical calculations, showed that the bands in this region are due to the torsional vibrations of pyronose rings. If the assignment is correct a strong dependance on inter and intra molecular hydrogen bonding is expected. Crystallization is found to have strong influence on band intensity.

From the low frequency infrared spectra it is observed that the band at 342 cm\(^{-1}\) which is prominent and sharp in crystalline cellulose I is reduced to a very weak band in decrystallised cellulose.

When cotton was treated with EDA and ethylamine the band at 1429 cm\(^{-1}\) decreased in intensity whereas the one at 893 cm\(^{-1}\) increased in intensity. This is treated as an indication of decrease in crystallinity by Pandey and Iyengar. They found similar behaviour during treatment with
alkalies. They also observed decrease in intensity of band at 1163 cm\(^{-1}\) and 1111 cm\(^{-1}\) on treatment of cotton with alkalies. The band at 1163 cm\(^{-1}\) has been assigned to the antisymmetric stretch of C-O-C bridge. Thus band has been used Iyer et al for analysis of wool/cotton blends.

Iyer et al have warned that a careful drawing of baseline for band at 1429 cm\(^{-1}\) and 893 cm\(^{-1}\) is necessary because of their steepness. A slight change in orientation of the base line will change the intensity of band to a great extent.

A new Infrared (IR) Ratio for measuring crystallinity of native cellulose based on absorption band at 342 cm\(^{-1}\) was developed at CIRCOT Bombay.

**SAMPLE PREPARATION:**

Infrared spectra are obtained on cotton fibre (cellulose) by taking 2.5 mg. fibre sample in the form of powder mixing with 200 mg. of alkali halide (KBr) and pressing the mixture into a pellet by applying a high load of 10 tonnes. The pellet had thickness of 1-2 mm. and constitutes a solid solution of sample substance (cotton fibre powder). Alkali halide KBr is used for preparation of pellets, of the solid material (cellulose, cotton fibres) under test. Because they are transparent to the infrared rays and they do not have selective absorption in the region of spectrum. Alkali halide (KBr) is hygroscopic hence sample preparation (pellet) was carried out in air-conditioned room at 45% (R.H.)
Spectro photometer - A Perkin-Elmer model-4 Infrared spectro photometer was used. It is double beam instrument. One beam from the infrared source passes through the sample and other is used as a reference beam. The two beams fall on a rotating semicircular sector mirror which reflects alternately the sample and reference beams on the slit of a grating monochromator. The dispersed beam passing through the exit slit falls on a thermocouple detector. If the sample absorbs at a particular frequency the two beams become unequal and the combined beam on the thermocouple detector flickers at a rate corresponding to the rotational speed of the sector mirror. The signal from the thermocouple which corresponds to the difference in intensity of the two beams amplified, rectified and given to a servo motor which moves an attenuator thereby producing infrared spectrum of cotton fibre.

Crystallinity is used fraction of OH groups, which are hydrogen bonded. Absorbances of OH – & OD – stretch bands at OH – 3360 cm\(^{-1}\) and OD – 2530 cm\(^{-1}\) are employed for this purpose.

O’connor employed an empirical crystallinity index ratio of absorbances at 1429 cm\(^{-1}\) and 893 cm\(^{-1}\). Band 1429 cm\(^{-1}\) attributed to CH2 bending mode decreases in intensity while 893 cm\(^{-1}\) resulting from vibration involving the C1 and the four atoms attached to it increases with decrystallisation.

Nelson and O’connor employed the band 1372 cm\(^{-1}\) as a basis for crystallinity determination. This band shows
Fig. 8

Schematic Diagram of
Perkin-Elmer 457
infrared spectrophotometer

1. source  
3. reference beam;  
5. grating monochromator;  
7. amplifier and rectifier;  
9. recorder;  
11. reference beam attenuator

2. sample beam;  
4. sector mirror;  
6. thermocouple;  
8. servo motor;  
10. scan motor;
comparable intensities in cellulose I and II and reduces in intensity only on account of decrystallisation. The ratio of absorbances at 1372 cm\(^{-1}\) to 2900 cm\(^{-1}\) could thus be applied for cellulose I and II and for samples containing mixed lattices. As for the first index, it appears that drawing base lines for peaks (1429 cm\(^{-1}\) and 893 cm\(^{-1}\)) could involve some uncertainty because the transmission intensities on the two sides of the peaks are highly uneven resulting in steep base lines. Band at 342 cm\(^{-1}\) which is prominent and sharp in crystalline cellulose-I, is reduced to a very weak band in decrystallised cellulose. Crystallinity index based on the absorbances of this band was found to be very useful in grading different samples of cellulose I structure. 1725 cm\(^{-1}\) band (C=O stretching) in polyester.

3.4 ECONOMICAL CHARACTERS:

3.4.1 BOLL WEIGHT (g):

Seed cotton yield obtained per plant was divided by number of matured and picked bolls per plant to obtain average boll weight.

3.4.2 SEED COTTON YIELD PER PLANT (g):

Every plant under observation was picked separately. Produce from good loculi of all pickings contributed to the seed cotton yield per plant.

3.4.3 GINNING PERCENTAGE:

The produce of five observation plants was bulked and a clean representative sample was drawn. The seed cotton was
ginned on mechanically operated gin and the weight (in gram) was taken for lint and seed obtained. The ginning percentage was calculated by using the formula.

\[
\text{Ginning percentage} = \frac{\text{Weight of lint}}{\text{Weight of total seed cotton}} \times 100
\]

3.4.4 LINT YIELD PER PLANT (q) :

The lint yield per plant was calculated by using the formula

\[
\text{Lint Yield} = \frac{\text{Yield of Seed Cotton} \times \text{G.P.}}{100}
\]

3.4.5 SEED INDEX (g) :

This is the weight in grams of 100 healthy seeds. A random sample of 100 healthy seeds was weighed to get the seed index in grams.