Materials and Methods

A research design after requisite validation was followed during the course of study. The multifaceted nature of the research problem called for analysis of different facets using specific methods and techniques. Multi dimensional approach was followed using both qualitative and quantitative methods of observation. The entire research program was divided into three components:

- The Ecological component-involving study of basic ecological principles in GMC cultivation and its coherence with existing agricultural system in India.
- The Social component- involving use of surveys, questionnaires and interviews to gauge the awareness as well as views of different sections of the society on this polemic issue.
- The Legal component- involving extensive study of Indian regulatory mechanism, its position at international level and global comparative study of GMC regulation.

Data Collection

1. ECOLOGICAL ANALYSIS
   Variation in the following components of Bt and non-Bt cotton plants were observed and assessed.
   - Field surveys- involving vegetation analysis, study of insect diversity, Plant morphology, Soil analysis
   - Experiments- conducted in Laboratory, Glass dome and open field.
   - SEM Analysis of Cotton Pollen and Cotton Fibre

2. SOCIOLOGICAL ANALYSIS
   Different segments of the society were surveyed to assess their knowledge as well as perception on GMCs.
   - Interaction- with people who have played influential role on decision making on this issue by personal interviews
   - Stakeholder survey- from different segments of the society using questionnaires.
3. LEGAL ANALYSIS

Detailed analysis of Indian Policy Framework and its evolution in the country was studied. Comparative global analysis of GMC regulation and its coherence with international protocols and legislation was sketched out.

- Analysis of Indian laws- involved identifying gaps between the existing regulatory framework and the underlying ecological principles.
- Overview of Global laws- on GMCs in different countries was drawn on the basis of the continental divide
- Study of International Legislations - was done to assess their coherence and role in trade and transboundary movement of GMCs and its products.

Analysis of Data

The qualitative and quantitative data was interpreted into substantive results based on systematic analysis. The quantitative data collected from different sources was analysed using befitting statistical tools. SPSS (Statistical Package for the Social Sciences) 16 was used for the same purpose. The qualitative data collected during personal interactions and legal review with underlying ecological principals was synthesized and reproduced into meaningful results.

1. ECOLOGICAL ANALYSIS

Set I Field Survey

Field survey of the Cotton growing North –Western belt of the country was done. Three survey sites were randomly selected in the states of Punjab and Rajasthan

- Site 1- Village Rupana, District –Mukatsar, Punjab.
- Site 2- Village Muradwala Dal Singh, District –Abohar, Punjab
- Site 3- Village Fatuhi, District –Ganganagar, Rajasthan

At every site two cotton growing fields one of Bt Cotton and other of Conventional Cotton were surveyed with prior permission of the landholder. In all total six field surveys were done at three different sites. A
field survey checklist (Annexure-I) was prepared outlining the various on-site experiments and sample collection to be done. Latitude and longitude of every site was recorded using Global Positioning System (GPS). Temperature and Humidity was also measured on-site.

Vegetation Analysis

Onsite vegetation analysis was done using the quadrat method. The minimum quadrat size was determined for every field. Fifteen quadrats were laid in the vicinity of every field for determining the presence of vegetation other than the crop. The relative density, relative frequency, relative abundance and relative dominance were calculated. The importance value index was also calculated for all the species observed. Finally the vegetation diversity in every field was measured using the Shannon Weaver Index.

Insect Diversity

The Net-sweep method was used to analyse insect diversity. Eight Sweeps each at randomly selected three different locations on the every field were done. All the readings were taken in the morning time 1000hrs. Data so collected was analysed to infer the insect diversity of that area using the Shannon Weaver Index ($H'$).

Plant Morphology

To study the plant morphology height, number of leaves per plant, leaf surface area, stem surface area and root volume were calculated on-site for the fresh biomass. For this purpose, twenty plants were randomly selected in every field and readings recorded.

Soil analysis

Soil sampling at every field was done in triplicate at three depths, 0-15cms, 15-30cms and below 30 cms. All the six samples collected from the six different fields were tested for the presence of macro and micro nutrients and the physio-chemical characteristics. Physio-chemical characters measured were pH, Electrical conductivity and organic carbon. Macro-nutrients N, P, K and micro-nutrients including Zn, Fe, Mn and Cu were measured in the soil samples.
Set II Experiments

The experiments were conducted using the only commercially grown GMC in the country i.e. Bt Cotton. Comparisons were drawn between Bt and non-Bt cotton plants. These experiments were carried out at three successive phases starting with lab experiments to Glass house experiments and finally in one kanal open field. The seeds used were procured from the farmers in cotton growing belt of Punjab.

Phase I Laboratory

The three varieties of cotton seed, *G. herbaceum* (Desi kapas), *G. hirustum* (American cotton), and GM *G. hirustum* (Bollgard II) were procured from the farmers in Mukatsar district of Punjab. The three seed varieties were grown in round petridishes of diameter 5” each and seed germination recorded. the experiment was aborted when till seven days no more germination was recorded and readings were taken for various parameters including radicle length, plumule length, root hair, and moisture content. The chlorophyll content of the seedlings was measured using DMSO method followed by spectro-photometric analysis.

Phase II Glass dome

The second level of experiments involved growing the three varieties of cotton in plots in a glass dome in the Botanical garden of the campus. The glass house used to grow three varieties of cotton in three beds. The row to row and seed to seeds distance was set at 40 cms in all the three beds. 21 seeds of each variety were tagged and sowed in each bed. The soil temperature and pH was recorded at the time of sowing.

For repetition, similar three beds were sowed in the open space in the botanical garden within the gap of 7 days maintaining same measurements. Experiments were then performed simultaneously for both the plots: Plot A in the glass dome and Plot B in open. Daily record of temperature, seedling emergence and plant growth was maintained. No fertilizers or pesticides were used during the course of experiment. The experiments performed have been categorized into four parts:
• Plant growth
The height, number of leaves per plant, stem surface area and surface area of leaf for 10 plants of each type in three replicates was measured and recorded.

• Study of worm infestation
To study the extent of pink bollworm infestation, number of worms per plant, number of worms per leaf, number of leaves infested and surface area of leaf infested was measured and recorded taking 10 as the sample size in three replicates for all three varieties.

• Forced worm infestation on Bt cotton leaves
To analyse the effect and efficacy of Bt-cotton resistance to bollworm infestation, an experiment of forced infestation was formulated and exercised. Under this experiment, living bollworms on the conventional cotton and Desi kapas were put in a fibre bag. This fibre bag was then tied on to the leaf of a Bt cotton plant in the plot. The experiment was repeated thrice on 10 different Bt-plants with 20 living worms in each bag. The bag was opened after 72 hours and number of living worms recorded.

• Soil Analysis
After aborting the experiment, soil sampling from the three beds growing three different varieties was done. As well as the rhizosphere soil was also collected from the roots of the three different plants to be analysed later. A control soil sample was also taken.

Phase III Open Field
The third level of experiments involved growing the plants on larger scale in the open field. For this purpose 1 kanal land was used in the village Tasauali, in district Mohali of Punjab. The American cotton and Bollgard II seeds were purchased at price of Rs. 450/kg and Rs. 1200/kg from local dealer. The land was ploughed, prepared and divided in two equal halves. The seeds were sown at approximately 20 cm row to row and seed to seed distance. The soil temperature and pH was recorded. Daily observation of the field and record of temperature was maintained. Record of irrigation, fertilizer used, pesticides sprayed was maintained.
The cotton yield of both the varieties was recorded and experimentation was done on following parameters.

- **Plant growth**
  The height, number of leaves per plant, stem surface area and surface area of leaf for different plants of both types were measured and recorded for 10×3 replicates.
- **Analysis of cotton yield**
  Number of cotton balls per plant for both varieties and weight of cotton collected at every picking was recorded. The biomass of the plant was also measured in comparison to the number of cotton balls.
- **Analysis of cotton lint**
  Moisture content of the cotton ball and rough length of the fibre was recorded for 10 plants in triplicate of both types.
- **Soil Analysis**
  After aborting the experiment, soil sampling from both the halves of the one kanal land was done at three depths along with control sample. Rhizosphere soil samples from both the types of cotton plant were also taken.

**Set III Scanning Electron Microscopy**

Scanning Electron Microscopy of the cotton pollens and cotton fibre was performed at the Central Instrumentation Laboratory, in the PU campus. The samples were collected on field and fixed in 2.5% glutaraldehyde. A comparative analysis of SEM images of Bt and Conventional cotton pollens and fibre was drawn.

### 2. SOCIOLOGICAL ANALYSIS

**Interaction**

It involved interaction with people who have played influential role on decision making on this issue was done by holding personal interviews. An open ended and a common questionnaire was prepared for the said purpose (Annexure-III). Individuals interviewed were sectionalized into three categories: Government Functionary, Expert Scientist and NGO Activist. Total of twelve individuals were interviewed.
Stakeholder surveys

Surveys for different segments of the society using questionnaires were performed. Six separate questionnaires were developed for different stakeholders of the society:

• Q Ia Common Man
• Q Ib Common Man -less educated
• Q II Retailer
• Q III Farmer
• Q IV Legislator
• Q V Scientist

All the questionnaires so prepared were pre-validated from any randomly selected members of the society before conducting the survey. Around 50 members were surveyed in each category with dual objective of gauging their awareness and views on the contentious issue.

3. LEGAL ANALYSIS

Analysis of Indian Laws

Government of India is ceased of the compulsion and limitations, as also the potential dangers of GMCs. For such reasons policy framework of the Ministry of Science and Technology (Department of Biotechnology) and Ministry of Environment and Forests, have enforced certain guidelines. In depth study of these guidelines evoking its strengths, weakness and flaws was done.

Overview of Global Laws

World over, evolution of the GMC legislation started simultaneously but quickly took different paths. The contrasting and divergent legislations between countries originate from their respective varying economic, political and social set up. A brief comparative analysis of GMC legislation form continent to continent was performed by a drawing a synthesis of the same.
Study of International Legislation

As GMCs appear to be great business opportunity for many countries, its trade between the countries is also prevalent. Such trade becomes arduous because of lack of regulatory concordance at global scale. Various international legislations pertaining to GMCs given by WTO, Codex Commission, FAO, WHO and CBD were studied and analysed for concordance.