INTRODUCTION
Hugo de Vries, the Dutch botanist, in 1900 coined the term ‘mutation’ for sudden hereditary changes in *Oenothera lamarckiana*, the evening primrose. But mutations were known to occur in animals and plants much before this time. For example, Seth Wright, an English farmer discovered a short legged sheep in 1791; this sheep was used to establish a breed named Ancon. Mutations may be chromosomal, cytoplasmic or gene mutations (point mutations). Mutations may occur spontaneously at a low frequency of one in 10 lacs i.e., $10^{-6}$, are usually recessive, deleterious in effect, random and recurrent in occurrence. Induced mutagenesis provides a base for strengthening crop improvement programmes by generation of genetic variability.

1.1 History of Mutation Research

Auerbach (1976) divided the history of mutation research into five periods. The first period was between 1900 to 1927. During this period the concepts of mutation and mutation rates were developed, the basic questions about the nature of mutation were formulated and techniques for measuring mutation rates were worked out. The discovery of the mutagenic action of X-rays in 1927 lead to the starting of the second period. X-rays provided a tool uniquely suitable for probing into the nature of mutation. The ‘target theory’ an unified theory of mutation had been constructed by the end of the second period in the late thirties. The target theory was that the genes and the presumed links between them are ‘targets’ for ‘hits’ by energy packets that are delivered to them by ionizing radiation. Point mutation is produced by a hit in a gene and a chromosome break by a hit in a link between genes. Just before the
second world war, the third period of mutation research started. At that time chemical mutagens became available. Also micro-organisms were introduced as a means of experimental analysis for studying the mutagenic action especially by ultraviolet radiation. A chemical model of the gene was necessary for interpretation of most of the findings of this period. Such a model was however not available till 1953 when Watson and Crick provided a structural model for DNA. The fourth period, from 1953 to about 1965, was dominated by studies on the chemistry of DNA and the action of mutagens on DNA. It was later realised that cellular mechanisms such as repair and expression, play important roles in the mutation process. In the present fifth period which started in 1965, the physical and chemical knowledge obtained during the preceding ones is being used as the basis for analysing mutation as a biological process.

In the early part of the 20th century, experiments conducted in Morgan’s laboratory using mutants of Drosophila lead to the realisation that the nature of mutation was inextricably linked with the nature of the gene. In 1927, H.J. Muller presented data at the Third International Congress of Genetics that demonstrated unambiguously the capacity of X-rays to produce mutations. The following year, L.J. Stadler demonstrated the same in maize. Subsequently X-ray mutagenesis dominated almost all branches of genetics for two decades. That UV could produce mutations in Drosophila and flowering plants had been established by the early thirties. Ionizing radiations such as Gamma were used for the production of mutations in agricultural plants from the end of 1930’s. In 1946, Auerbach and Robson demonstrated that mutations could be produced by nitrogen mustards in Drosophila. Although this was the first application of a chemical mutagen, several others became available later on and found widespread application in inducing mutations.
1.2 Nature of Mutations

Mutations are of different types. Mutations produced by changes in base sequence of genes as a result of base pair transition or transversion, deletion, duplication or inversion etc. are known as gene mutations. Those produced by changes in chromosome structure or even in number are termed as chromosomal mutations. Gross chromosomal changes such as changes in chromosome number, translocations, inversions, large deletions and duplications are detectable under the microscope. In cytoplasmic mutation, the mutant character shows cytoplasmic or extranuclear inheritance. Bud mutations or somatic mutations occur in buds or somatic tissues which are used for propagation, e.g. in clonal crops.

New alleles are rarely produced in induced mutations but they produce alleles which are already known to occur spontaneously or may be discovered if an extensive search is made. The effects and the variability produced by induced and spontaneous mutations are comparable. The great advantage of induced mutations over the spontaneous ones is that they occur at a relatively higher frequency so that it is practical to work with them. Mutations have certain general characteristics which are summarised below:

1. They are generally recessive, but dominant mutations also occur.
2. A small portion (0.1%) are beneficial but a majority of mutations are generally harmful to the organism.
3. Mutations may occur at random in any gene. However, some genes show higher mutation rates than the others.
4. The same mutation may occur again and again, that is, they are recurrent.
1.3 Effects of Mutation

In general, mutations have harmful effects on organisms. The viability of the individuals that carry mutations is usually reduced. Mutations are classified into four groups, based on their viability. They are:

**Lethal mutations**: This type of mutation normally kills all individuals that carry them. Dominant lethal mutations affect even heterozygous individuals, while recessive lethals kill only the individuals which carry them in the homozygous state.

**Sub lethal and Sub vital mutations**: Do not kill all the individuals that carry them but reduce the viability. Sublethals kill more than 50% of the individuals while subvitals kill much less than 50%. This type of mutations are of no value although a vast majority are of these type.

**Vital mutations**: The viability of the individuals carrying this type of mutation is not reduced. This class of mutations occur in a much lower frequency than the other three types, but are the only ones that can be utilised in crop improvement.

1.4 Induction of mutations

Treatments with certain agents known as mutagens can be used to induce mutations at relatively higher frequencies. Mutagens may be different kinds of radiations (physical mutagens) or certain chemicals (chemical mutagens).

1.4.1 Chemical Mutagens

These are broadly classified into:
1. Alkylating agents, for instance
   Sulphur mustards
   Nitrogen mustards
   Epoxides
   Ethylene imines (EI) e.g. Ethylene imine
   Sulphates and Sphonates, e.g. Ethyl Methane Sulphonate
   (EMS), Methyl Methane Sulphonate (MMS)
   Diazoalkanes
   Nitroso Compounds, eg. N’-methyl-N-Nitro-N-
   nitroso-gunanidine (MMNG)
2. Acridine dyes, eg. acriflavine, proflavine acridine orange, acridine
   yellow, ethidium bromide
3. Base analogues, eg. 5-Bromouracil, 5-Chlorouracil
4. Others, eg. nitrous acid, hydroxyl amine, sodium azide

**Mechanism of action of chemical mutagens**

The mechanism of action of each group of chemical mutagens is different. A mutagen may produce mutagenic or inactivating changes in DNA. While a mutagenic change does not prevent replication, it produces changes in one or more nucleotides and does not induce chromosomal aberration. Such changes are the results of changes in hydrogen bonding properties of bases or mistakes in base pairing during DNA replication. Inactivating alterations prevent DNA replication across the altered site, induce chromosome breaks and chromosome mutations. The inactivating alterations of DNA in most of the cases are repaired by cellular enzymatic repair mechanisms.
1.4.2 Physical Mutagens

These include various kinds of radiations. Such as

1. Ionising radiations
   a. Particulate radiations
      α-rays (Densely ionizing)
      β-rays (Sparsely ionizing)
      Fast neutrons (Densely ionizing)
      Thermal neutrons (Densely ionizing)
   b. Non particulate radiations (Electro magnetic radiations)
      X-rays (SI)
      γ-rays (SI)

2. Non ionizing radiations
   UV radiation

X-rays and γ-rays

X-rays and γ-rays are similar in physical properties and biological effects but they differ in the source of their origin. X-ray tubes are used to produce X-rays while radioactive decay of certain elements produce γ-rays, e.g., radium, ¹⁴C, ⁶⁰Co etc. The common source of γ-rays used for biological studies is ⁶⁰Co. Depending upon the wavelengths, X-rays are often referred to as hard or soft. X-rays are high energy radiations (small packets of energy) and are sparsely ionising. They produce Photoelectric Effects, Compton Scattering and Pair Production.

UV radiation

UV is a low energy radiation, does not cause ionisation and has a very limited penetrating capacity (one or two cell layers). It is present in solar
radiation and can also be produced artificially by Mercury vapour lamps or tubes. Generally, UV rays produce dimers of thymine, uracil and cytosine present in the same strand of DNA. It also produces addition of a molecule of water to the 5,6 double bond of uracil and cytosine, which promotes deamination of cytosine. The dimer formation and deamination are likely to be the reason for the mutagenic action of UV. In micro-organisms, UV is commonly used to induce mutations since penetration presents no problem in that system. Use of UV in higher organisms is limited to irradiation of pollen grains (in plants) and to small eggs (e.g. in Drosophila).

**Mechanism of action of radiations**

Radiations are direct as well as indirect in their effects. Energy is transferred directly by the radiation to a molecule in direct effect but in indirect effect it is mediated by free radical formation: the radicals transfer their energy to other molecules. The indirect effect is particularly important in presence of water since ionised water molecules produce free radicals (Singh, 1983).

According to Evans (1962) radiation does not produce direct breakage in chromosomes, but initiates a lesion requiring DNA synthesis for repair. An exchange would arise as a consequence of misrepair of the lesions. Revell (1959) have stated that all aberration are a consequence of exchange following a process of misrepair of primary lesions. Variations in radiosensitivity at different stages of the cell cycle are due to differences in the time available for repair and to changes in chromatid structure during chromosomal replication.
Radiations have been extensively utilised for many years to cause mutations and chromosomal damage for experimental purposes. They can induce a change in the molecular organisation of protoplasm. The change may be expressed as a mutation, a break in a chromosome, or an alternation in the physiological activity of the cell (Cohn, 1969). The manner in which the yield of structural changes increases with increase of the dose of radiation has been extensively studied, and the results of these studies form the main basis on which theories of the mechanism of induction of these changes are built (Lea, 1946).

1.5 Effect of light in the visible range on plants

A number of processes in plants such as photosynthesis, protoplasmic streaming, flower induction, seed germination, chlorophyll biogenesis, bending of organs and numerous other growth reactions are affected or controlled by radiant energy. Under optimum conditions these processes are normal. But variations above a certain range induces stress symptoms in plant (Noggle and Fritz, 1983). Radiation between 510 and 610 nm (green yellow) has minimal effect on plant growth where as radiation between 400 and 510 nm (blue) will have the following effects - chlorophyll and other photosynthetic pigments such as phycocyanin, phycoerythrin and carotein have peaks in absorption in these wavelengths. Phototropic movements of plants are promoted by absorption of radiant energy of these wavelengths. It has been thought that visible radiation will not be mutagenic since most of the biological molecules have optical absorption in the UV region. Since the discovery of laser in 1960, the idea that the visible light may be mutagenic has been mooted. Putative mutagenicity has been attributed to the coherence and
intensity of laser beams, due to which it can interact with biomolecules in a non-linear way.

Laser is an acronym for Light Amplification by Stimulated Emission of Radiation. Laser beam consists of photons having associated wavelengths that are exactly in phase, hence the term coherent. Laser beam is highly directional and due to this property it is extremely bright i.e., power per unit area per solid angle is very high and it is monochromatic. Lasers are produced by exciting an absorbing material with electromagnetic radiations. Lasers are classified as gas lasers, dye lasers, semiconductor lasers, etc. In the case of gas lasers, pumping is caused by passing an electric current. Examples for gas lasers include Helium-neon laser, Argon ion laser, Krypton ion laser etc. There are infrared, visible and ultraviolet lasers. Visible lasers normally function in wavelengths between 400-600 nm.

Modern laser techniques provide a wide range of variation of radiation parameters such as frequency, intensity and pulse duration thus making it possible to carry out investigations on selective action on substances. Molecules or part of molecules of the same type may undergo considerable change caused by photoionisation or photodissociation with subsequent chemical reactions.

**Objectives of the present study**

Genetic improvement of crops is dependent on the availability of genetic variability. Sources to induce such variability include the use of physical and chemical mutagens, tissue culture etc. Although lasers have recently been suggested to be useful in inducing mutations, their use has been limited in the...
absence of any systematic study to establish their precise mutagenic nature. In recognition of this, the specific objectives of the present study were as follows:

1. To study the effect of laser radiation in inducing chromosomal aberration during mitotic cell division by analysing the root tip squashes of two plant species - *Vicia faba* and *A. cepa* L. in comparison to the effect of physical mutagens such as γ-rays and UV-rays, and chemical mutagens such as Ethyl Methane Sulphonate (EMS) and Hydroxyl Amine (HA).

2. To compare the effect of laser radiation and the aforesaid mutagens on mitotic index in the two plant species.

3. To study the effect of laser radiation on meiotic cell division in the flower buds of *V. faba* in comparison to the effect of the other mutagens.

4. To compare the pollen grain sterility in these different treatments.

5. To study the effect of laser radiation and the other mutagens on growth and yield in the two plants species.

6. To study the effect of laser irradiation on enzyme activity, specific activity and soluble protein content in comparison to the effect of EMS and γ-irradiation.