Introduction

1.1 Mutagenesis

Mutagenesis is described as the exposure or treatment of biological material to a mutagen, i.e., a physical or chemical agent that raises the frequency of mutation above the spontaneous rate (Reiger and Green, 2012). Induced mutagenesis is the method by which DNA mutations are intentionally engineered to produce mutant genes, proteins or strains of organisms. The purpose of mutation breeding is to improve the locally adapted varieties by creating new alleles not available in germplasm collections (Solanki et al., 2011). Hugo de Vries in 1900 used the term “mutation” to describe changes that are inheritable. Thus, a mutation can briefly be defined as a sudden heritable change in the DNA of a living cell not caused by the common phenomenon of genetic segregation or recombination. The mutants themselves may not be suitable for direct release, but they do provide the necessary alleles for developing superior cultivars with desirable traits. Induced mutations are helpful to smash undesirable linkage between two characters. It has been observed that induced mutations can increase yield as well as other quantitative traits in plants (Dhulgande et al., 2010).

1.1.1 Starting Plant Material

The seed is the most commonly treated biological material because it can tolerate physical conditions which generally are tolerated only by non-living molecules (Solanki et al., 2011). Usually multicellular tissues are used as the starting material for mutation induction.

1.1.2 Reproductive Biology of the Crop

Knowledge of the reproductive biology of the crop, self-vs. cross fertilization, facilitates ease of propagation methods possible (in vivo vs. in vitro). As each crop spp. has a variable reproductive capacity (number of progenies per plant) and a specific system of reproduction (self or cross pollinated sexual reproduction or asexual propagation a
universal breeding approach cannot be developed and specific procedures have to be
applied.

1.1.3 Selection of Variety

Since genotypes give differential response to mutagens, two or more varieties must be
taken for mutagenesis. It is always beneficial to select a well-adapted high yielding
variety for improving one or two specific traits (Anonymous, 1977).

1.1.4 Population Size

The length of exposure and the type of tissue determine the extent of mutation induced.
Often large populations are needed to warrant that the desired gene bears a significant
percentage of mutations. Most mutagenized populations are generated by exposing seeds
(M₀) to the mutagen and allowing the resistant M₁ plants produced to self-fertilize and
give rise to M₂ seed. To ensure the greatest number of unique novel mutations it is
recommended that only one seed is taken from each M₁ plant. However at this stage, the
population is still segregating and not all M₃ plants will carry the mutation identified in
the M₂ parent. The mutagenized population may be taken through further generations by
single seed descent to generate near homozygous material (; 3% heterozygous mutations
at M₆), although up to half of all mutations present in the M₁ are lost in the process. Some
thousands of seeds are usually needed, being aware that the handling of such large
populations requires efficient mass screening techniques. When planting seeds in soil,
emergence is taken as the criterion instead of germination. Selection starts in the
segregating M₂ population or in the M₃ for traits that can be screened for only on a row
base. Dominant mutations, which are very rare, can be selected in M₁ already (Parry et
al., 2009).

1.1.5 Dose Selection

For mutation induction, it is advisable to use two to three doses along with a control
(Kamra, 1997; IAEA, 1985). The dose can be defined as a particular mutagen
concentration for a definite period of time at a particular temperature. A pilot assay is
usually necessary in order to determine the optimal dosage prior to large scale bulk
treatment of propagules (Mba et al., 2010).
The dose of mutagen that is regarded as the optimal is one that achieves the optimum mutation frequency. The LD$_{50}$ is an important parameter to measure the short-term poisoning potential (acute toxicity) of a treatment and widely used to determine the optimum mutation frequency with least possible unintended damage (Owoseni et al., 2006). Meyer (1996) described that the LD$_{50}$ is given at once and causes death of 50% of the test material. The mutagen dose mainly depends upon the concentration, duration of treatment and temperature during the pre-treatment. Pre-soaking, pH of solution, metallic ions, carrier agents, post washing, post drying and storage of seeds are the modifying factors for the mutagenic effect (Solanki et al., 2011). Mba et al. (2010) provided the protocols for the induction of mutations in seed and vegetatively propagated plants, based on procedures validated for rice and cassava respectively. The protocols were for the physical mutagen (gamma rays generated by a cobalt-60 source) and the chemical mutagen (EMS). The universally adopted norm is to select a dosage that results in reductions of 30 to 50 or 40 to 60 % in growth or survival rates, respectively of the first generation mutant (M$_1$) seedlings compared to the seedlings of untreated seeds. Reductions in germination rate, seedling height, and survival rate, no. of tillers, seed set and fertility test in the M$_2$ generation as well as chlorophyll mutation are the main parameters measured in the sensitivity tests for determining the optimal doses.

1.2 Types and Efficiency of a Mutagen

Agents that are used to induce hereditary changes are broadly divided into physical and chemical mutagens, and the most enviable mutagen is the one that is least damaging and a high mutation yielder (Kaul, 1989). Chemical mutagen is a mutation agent which is in the form of chemical substance. It can mimic nitrogen base in normal DNA but they cannot couple during DNA replication. Moreover, chemical mutagen has an ability to insert between nitrogen bases and disturb DNA replication. Physical mutagen, on the other hand, is a mutation agent which is in the form of physical substances, such as short wave (ultraviolet and radiation ray such as alpha, beta, and gamma). Some physical mutagens can cause ionization while some others cannot.
A quick and simple method to evaluate the mutagenic effect is to determine the primary injury in M₁ seedlings under greenhouse conditions. Primary injury includes reduction in seedling height, root length, survival, and fertility.

1.2.1 Advantages and Limitations of Induced Mutagenesis

Induced mutations are playing a major role in basic studies especially for the elucidation of biochemical and plant developmental pathways. For example identification of key genes involved in floral development which ultimately led to the construction of ABC model of flower development, was made possible through the isolation and molecular characterization of floral mutants of Arabidopsis and Antirrhinum (Coen and Meyerowitz, 1991).

A specific advantage of mutation induction is the build up of a spectrum of mutant lines and discovery of the trait-specific genes to establish a molecular gene database, study molecular functional genomics and develop bioinformatics for future to develop plant variety. Mutant lines (i.e. lines carrying foreign DNA insertions into host chromosomes) can be isolated on the presence of foreign DNA sequence.

Another promising feature is the isolation of multiple trait mutants in contrast to the transgenic approach where single trait can be introduced in the crop and moreover modified (GM) food has garnered less approval among masses. Mutagenesis cannot be deemed as gene maneuvering because the base alterations are analogous to those occurring naturally.

Plants incompetent for cross breeding due to sterility can have their genetic variability enhanced by induced mutagenesis is a short time span.

The frequency of induction of loss of function mutations is much higher than those ameliorating the beneficial traits associated with the targeted gene. Raising large mutant populations, the incidence of chimeras and heterozygosity of the mutated loci are major deterrents of induced mutagenesis. The largely recessive nature of the mutations masks the expression of the desired phenotype if the alleles exist in a heterozygous state.

The main handicaps of mutation breeding are the negative selection value and the
pleiotropic action of mutant genes selected. If a mutant shows a positive character which might be of interest for plant breeding, it shows very often, one or several less negative traits making it useless for breeding purposes. Despite an array of mutagenic agents at our predisposal it is still an arduous task to manually direct mutations exhibiting the intended expression of characters.

1.2.2 Considerations

Before starting the mutation breeding programmes the following points should be taken into consideration—

1. Dominant mutations occur at very low frequencies and can be selected in M₁ (Micke and Donini, 1993).

2. Mutations are predominantly recessive and can be selected in the second generation. (Salimath et al., 2007; Toker et al., 2007, Toker et al., 2011).

3. Selection for polygenic traits should be initiated at individual plant progenies in M₃ (Micke and Donini, 1993; van Harten, 1998; Toker et al., 2011) Polygenic traits (i.e. characters under the influence of many genes) have less chance of being modified than monogenic traits (i.e. characters under the influence of single genes).

4. Mutations are beneficial with very low frequencies, whilst mutagenic treatments by and large reduce germination, growth rate, vigour, pollen and ovule fertility in living organisms (Toker et al., 2011).

5. Mutations are randomly induced and they might occur in any gene(s). However some genes can be more induced than others (Toker et al., 2011).

6. Mutations may be recurrent. The same gene(s) in a crop plant species may be induced again.

7. Mutations have generally pleiotropic effects due to closely linked gene(s) (Singh, 2005; Salimath et al., 2007; Toker et al., 2007, Toker et al., 2011).
1.3 Objectives

❖ To study the cytology (mitosis and meiosis) of *Crotalaria juncea* L.

❖ To screen out the effect of physical mutagen *viz.* gamma rays on *Crotalaria juncea* L. and evaluate their cytomorphological impact.

❖ To analyze the effect of certain chemical mutagens *viz.* EMS and MMS on cytology and morphology of *Crotalaria juncea* L.

❖ To study the effect of colchicine on the induction of polyploidy in *Crotalaria juncea* L.

❖ To study the effect of heavy metals (Pb,Cd) on the cytology of *Crotalaria juncea* L.

❖ To evaluate the number of mutants and variants on the basis of variations in qualitative and quantitative trait.

❖ To study the effect of mutagens employed on all the morphological parameters of the plant.

❖ To study the changes in biochemical parameters in response to induced mutagenesis.

❖ To study DNA polymorphism in induced mutants/variants with respect to control using universal primers.

❖ Fibre analysis of gamma irradiated set of induced mutagenesis.