Chapter - I

INTRODUCTION

Pea (*Pisum sativum* L.) is a cool season annual crop produced worldwide for human consumption and animal feed. The garden pea is widely cultivated and grouped as *Pisum sativum* L., subspecies *hortense* and the field pea as *Pisum sativum* L., subspecies *arvense.* Edible podded peas are found in both these subspecies. **Govorov (1928)** suggested the inclusion of all cultivated forms of peas in one species *Pisum sativum* L., and subdivided it into two subspecies *viz*., *sativum* L. and *arvense* L. **Menjkova (1954)** observed that *Pisum sativum* and *Pisum arvense* crossed readily and gave fertile progeny and normal segregates. He concluded that the two kinds pertain to the same species. Both of these have the same chromosome number 2n= 14 **(Dhulgande *et al*., 2011)**.

The consumption of pulses with cereals is a common practice in India and this practice is considered to be very scientific because of high protein content in pulses, which is required for balanced food as a large population in the country is dependent upon vegetarian food. It has been recommended that balanced protein nutrient should constitute 70 per cent and 30 per cent dietary protein from cereals and pulses respectively. The pea varieties are of three seed types *viz*., round, dimpled and wrinkled. Early cultivation of pea was for pulse purposes and mostly round seeded varieties were grown mainly as a one word crop. The vegetable/ garden pea varieties are sweet in taste and hence are mostly wrinkled because of high sugar content. Peas are sown in *rabi* season from beginning of October to end of November in northern plains as the cool climate of about four month is ideal for pea growing. The areas where there is slow transition from cool to warm weather are ideal for pea growing. The optimum temperature for seed germination is about 22^0 C however; it can germinate up to 5^0C but at slow rate. The wrinkled seeded cultivars are more sensitive to high temperature and a temperature of 30^0C and above even for a day affects the quality of pods. Based on maturity it is grouped into three categories *viz*., variety taking less than 40 days to flowering (early), less than 55 days (medium) and late varieties taking more than 55 days to flowering **(Kalloo *et al*., 2005)**.

Pulses are important constituents in the diets of a very large number of people, especially in the developing countries and are good sources of protein which help to supplement cereal diets, improving their nutritive values. Pulses contain the same amount of calories as cereals but protein content is twice that of cereals. Pulses are mainly deficient in sulphur containing amino acids like methionine, but high in lysine content which is the most limiting amino acid of cereals. Some pulses are also deficient in tryptophan. Thus, cereal proteins deficient in lysine and pulses with adequate lysine content have a mutually supplementary effect, if both are consumed together. In addition to protein, carbohydrates are another major component of legumes. They also supply vitamins, minerals and fat. The presence of different types of proteins and other smaller molecules, including alkaloids, is flavones, polyphones and a variety of oligosaccharides, make legume unique in providing nutraceuticals. Fresh peas contain (per 100g) 44 calories, 75.6% water, 6.2g protein, 0.4 g fat, 16.9 carbohydrate, 2.4 g crude fiber, 0.9 g ash, 32 mg Ca, 102 mg P, 1.2 mg Fe, 6 mg Na and 350 mg K **(Singh, 2002).**

Presently, India produces around 14.66 million tonnes of total pulse from an area of 24.54 million hectares of land and yield 597 kg/hectares. The major pea growing states in India are Uttar Pradesh, Madhya Pradesh, Bihar and Maharashtra. Uttar Pradesh ranks first in area (0.4 million hectare) and about 90% of its area and production is limited to Uttar Pradesh alone **(Anonymous, 2013)**.

Pulses are unique crops in the sense that they constitute a group of leguminous crops, bearing the characteristics of root nodules under inbuilt mechanism to fix atmospheric nitrogen in symbiotic association with bacterium called *Rhizobium*, and by using *Rhizobium* in pulses crops, one can significantly minimize the application of nitrogenous fertilizers in boosting the production and improving the nitrogen status of soil.

The crop however, has sufficient genetic variability, it suffers from severe susceptibility to different biotic stresses, plant type, which is directly related to yield potential and disease resistance, has not been fully exploited in case of pea **(Moot and McNeil, 1995).** Induce mutagenesis has been successfully used to generate wider variability, particularly for isolation of mutants having desirable legume crops **(Kharkwal *et al*., 2004).** Gamma irradiation has been used extensively as a potential physical mutagenic agent **(Solanki and Sharma, 1994, Kumar *et al*., 2007)**. Improvement in the frequency and spectrum of mutations in a predictable manner and thereby, achieving desirable plant characteristics is an important objective of mutation breeding programme. Therefore, selection and subsequent application of effective and efficient mutagens (s) either singly or in combination is very essential to recover a high frequency and spectrum of desirable mutations **(Solanki and Sharma, 1994).**

Mutation breeding is relatively a quicker method for improvement of crops. It has been observed that induced mutations can increase yield as well as other quantitative traits in plants.

The choice of mutagen holds great importance in changing the frequency and spectrum of chlorophyll mutations in a predictable manner. The physical and chemical mutagens causes three types of effects i.e., physical damage, gene mutation and chromosomal aberrations **(Swaminathan, 1965)**.

Improvement of the cultivated plants largely depends on the extent of genetic variability available within the species. Variability observed in the existing germplasm collection owes its initial spontaneous mutations that were able to survive under specific environmental conditions and their subsequent recombination but with a very poor frequency (10^{-7}). In accelerating the pace of increasing variability the role of mutagenesis as a modern device is highly appreciated. Natural selection played a major role for its improvements than human selection in determining the morphological, physiological and agronomical characters. Induced mutations can be used to ratify simple, specific undesirable traits of well adopted varieties without disturbing its genetic constitutions **(Makeen, 2009)**.

Gamma radiation can be useful for the alteration of physiological characters. Mutagenesis by means of gamma rays has played an important role in the producing new mutants with improved properties which can produce higher amounts of commercially important metabolites the stimulatory effect of high dose in due to the fact that mutagens stimulate the role of enzyme and growth hormone responsible for growth and yield. Slow seed germination, limited and delayed rooting of the vegetative cutting has slowed down the propagation of this plant increased number of fruits per plant as a result of gamma irradiation was recorded **(Dubey *et al*., 2007** and **Mishra *et al*., 2007)**.

Mutation breeding methodologies need to be standardized for improvement of progenies traits to gain general confidence and wide acceptance of its usefulness among the breeders so that experiments could be planned with reasonable expectation of success. Broad spectrum genetic variability is a prerequisite for any successfull breeding programme. Attempts to induce mutations in pea would be quite useful in genetic variability. Besides, the use of induced mutations in fundamental studies, it can be used to create additional genetic variability for quantitative traits **(Swaminathan, 1968).**

In mutagenesis, the choice of the mutagen holds great importance in changing the spectrum of mutation in a predictable manner. Thus, main aim of this research is directed mutagenesis in cultivated crops. Mutations breeding technique has been used for the improvement of crops by creating new variability in desired direction, such as plant type resistance to biotic and abiotic factors with superior biochemical composition and better adaptation. Mutations are grouped into two major categories on the basis of their phenotypic manifestations **(Singh, 2009)**:

(i) Micro mutations – these involve changes in quantitative traits and can be measured at the level of population using various statistical parameters, such as character mean, variance, heritability etc.

(ii) Macro mutations – with large changes in the characters which can be detected even without instrumental help at the level of individual plant.

Screening technique to identify mutations that occur with very low frequency among a large number of traits has been advancement. The attempts have been made to develop standard screening technique for identification of micro mutations affecting the polygenic system. The work on comparative mutagen efficiency has been reviewed by **Kawai, (1969)** for oligogenic and polygenic characters and chromosomal aberrations. In general, selection for quantitative traits such as yield should be favorably carried out in early generations because most of the desired combinations of desirable alleles are likely to be lost in advanced generations due to intensive selection or without selections of other traits **(Sneep, 1977)**. However, after the studies of **Brock, (1967)**, it becomes a common practice to advance only normal looking M_2 plant in M_3 and M_4 generations. Whereas, the selection in M_3 generation results in an increased volume of non mutated materials, and delayed the isolation of promising variants. Consequently, the proclaimed advantages of rapid progress in breeding through mutation as compared to hybridization was lost and if also discouraged the worker is to adopt micro mutation as a tool for plant improvement. Some experiments demonstrated that selection in later generation is more effective than in M_2 generation **(Jana and Roy, 1973).**

Later on research were conducted to explore the possibility of selecting desirable plants for polygenic variability in earlier generation in M_2 **(Scossiroli, 1968; Sharma, 1977; Bhadra**, **1982)**. These workers concluded that promising progenies could be identified with high degree of precision in M_2 generation on the basis of mean and variance. This may be because of the fact that the materials already selected in M_2 may be confirmed with higher probability in subsequent generations **(Ravi *et al*., 1980).** Thus, the materials selected on later generation have higher probability of getting fixed as promising strain. There is no evidence, which suggests that the frequency of promising mutations *per se* over the entire population is higher in M_4 than in M_3 generation.

Moisture stress is a sustained period of time without significant rainfall **(Linsley *et al*., 1959)**. Whereas, **Quizenberry (1982)** suggested that such rainfall deficit does not constitute drought in a crop production system until the water scarcity begins to limit the growth and development of crop plants. At genetic level, the adaptive mechanisms by which plants survive drought, collectively referred as drought tolerance **(Jones *et al*., 1980)**, and grouped into three categories, *viz*., drought escape, drought avoidance and drought tolerance **(Leonardis *et al*., 2012)**.

Moisture stress is a complex character, expression of which depends on accomplishment and interaction of various morphological traits *viz*., earliness, reduced leaf area, leaf molding, wax content, efficient rooting system, stability in yield and number of branches; physiological traits *i.e*. transpiration, water-use efficiency, stomatal activity and osmotic adjustment and biochemical traits *i.e*., accumulation of proline, polyamine, trehalose etc., increasing of nitrate reductase activity and storage of carbohydrate. Very little is known about the genetic mechanisms that have room for these characters **(Kumar *et al.*, 2012)**.

The identification, inheritance and action of gene responsible for morphological and physiological traits in some crops have been reported. Root characters are inherited polygenically **(Ekanayake *et al*., 1985)** where the dominant alleles that govern long and more numbers of roots while; thick root tip is governed by recessive alleles **(Gaff, 1980)**. Leaf molding **(Turner, 1979)** and osmotic adjustment **(O' Toole and Moya, 1978)** have revealed monogenic inheritance. In cowpea, drought tolerance is reported to be governed by a single dominate gene **(Mai Kodomi *et al*., 1999)**. Potential sources of moisture stress species and genotypes of major vegetable crops have been identified in many of the vegetable crops. **Ronde and Spreeth, (2007)** used gamma rays irradiation to obtain a high frequency of gene mutation and chromosomal alterations. Irradiation dosages between 0 and 300Gy were applied in order to determine the optimal irradiation dose of 180Gy. Mature plants of M_1-M_4 generation were screened in a rain-out shelter and physiological traits for drought stress were identified. Roots of mature plants were also assessed and the variation observed could be correlated with drought tolerance. Six mutant cowpea lines were included in a physiological screening experiment that was conducted on greenhouse plants.

The application of mutagenesis in agriculture for improving the crop plants presented a new departure from the conventional breeding methods. In conventional breeding methods, the store of natural variability present either in the base population initially or introduced through hybridization, is subjected to recombination and selection so as to increase the frequency of favorable combinations of genes in the selected line. Mutation breeding helps in inducing greater magnitude of variability in various plant traits in a comparatively shorter time. Only through a careful screening and selection programme the magnitude of genetic variability induced by physical and chemical mutagens could be exploited for obtaining the desirable lines. Mutations provide an opportunity to create hitherto unknown alleles so that the plant breeder does not remain handicapped because of limited allelic variation at one or more gene loci of interest **(Bashir, 2012).**

Seed which is the basic and vital input for increasing productivity, losses vigour and viability during storage under improper conditions. Though, this is an inevitable phenomenon, it can be effectively controlled by physical as well as chemical means. Maintenance of high standards of seed germination and vigour from harvest until planting is at most importance in a seed programme. Seeds become practically worthless if fail to give adequate plant stand of healthy and vigorous plants on planting. Good seed storage is, therefore a basic requirement in seed production. This is particularly shown under inimical environmental conditions which would lead to the development of either hard seeds or off colour seeds in pulses **(Shukla and Prasad, 2009)**.

Seed storability is greatly influenced by two major factors *i.e.*, the relative humidity and temperature. It is therefore, more economical and easier to restrict moisture absorption by the seed either by dehumidification of the stores or by manipulating the packaging material used for storage. Keeping above consideration in view, the present study was undertaken with the following objectives.

(1) To estimate genetic variability for different quantitative and seed qualitative characters in induced population.
(2) To determine effectiveness and efficiency of mutagens used.
(3) Assessment of characters association and their effect on seed yield in induced population of pea.
(4) To assess the effect of packaging on seed quality parameters (seed viability and vigour) of gamma rays induced pea mutant after storage at different time intervals.