DISCUSSION

The major interest in mutation research on crops is the large genetic variation that it creates. Induced mutagenesis is an important technique for crop improvement in self pollinated crops, conventional methods of plant breeding practised ever since, scientific approach of plant cultivation was adopted, which dependent solely on the spontaneously arising genetic variability following the discovery of various mutagens and their usefulness. This offers possibilities for selection well beyond the limits which occurs in natural populations. In pea (*Pisum sativum* L.), induction of useful mutations has gained wide interest of the potential of this method for creating genetic variability. Pea being an important pulses crop with high nutritive value is also ideally suited for mutation breeding with a high probability of altering and improving any morphological character through induced mutation. The present study was therefore taken up to obtain theoretical and practical information on mutation induced in pea cultivar, namely Arkel by gamma rays effect of that mutagen. The magnitude of induced variability in pea after exposing the seeds to gamma radiation doses was estimated. In the initial phase of mutation breeding research, main attention was directed towards mutations of early identifiable characters, commonly referred to as macro mutations. The response of mutagenesis was measured through three generation: (I) M_1 generation, to estimate the effect of mutagens on physiological parameters and seed qualities parameters, (II) M_2 generation for macro mutational selections, (III) M_3 generation to test character stability.

5.1. Effect of mutagenic treatment in M_1 generation

Physiological damaged and chromosomal mutation caused by the physical mutagen in the biological materials could be measured quantitatively by the degree of reduction in germination, survival, seedling growth and pollen sterility.

5.1.1. Germination and plant survival

The experimental results revealed that there was a general reduction in seed germination and plant survival with the increasing different doses in M_1 generation. The **Table 4.1** showed that germination and survival has been reduced in higher doses as compared to lower ones. A reduction in germination and survival in the M_1 generation due to mutagenic treatment has been also reported in pulses by **Kumari and Singh, (1996), Ciftci *et al.*, (2006), Govardhan and Lal, (2013)** in pea, **Barshile *et al.*, (2006)** in chickpea, **Makeen *et al.*, (2007)** in urdbean, **Kavithamani *et al.*, (2008)** in soybean **Anbarasan *et al.*, (2013)** in sesame and **Ramya *et al.*, (2014)** in black gram.

Sato and Gaul (1967) considered that the reduction in survival and germination per cent could be attributed to chromosomal or physiological disturbances. Physiological damage, chromosomal aberrations and point mutations may be the cause of reduced germination and survival percentage in M_1 after treatment with physical mutation **(Gaul, 1970)**. Drastic reduction in germination and plant survival at doses is primary due to high frequency of chromosomal aberrations or altered physiological imbalance caused by mutagenic treatments.

Germination and plant survival involve a very critical growth phase which requires a proper metabolic state of affairs operative during seed germination and seedling growth; it also requires a perfect eco-physiological condition with the genetic components. All these biochemical process should remain under a particular state of equilibrium for germination and seedling growth. If any interference or disturbance occurs to this redesigned biochemical process during germination its adverse effect reflects on germination and plant survival. The reduction in germination and plant survival in M_1 generation is due to the effect of the physical mutagen such as physiological damage, chromosomal and gene mutations in the biological material could be measured qualitatively by the reduction in germination and survival.

5.1.2. Seedling height

The data on root and shoot length was recorded on 8 days after keeping the treated seeds along with untreated (control) seeds for germination. The data on seedling height are presented in **(Table 4.2)** revealed at seedling height in M_1 generation, medium dose (15kR dry) stimulate the seedling height and at high doses inhibits seedling growth.

M_1 seedling growth is widely used as an index in determining the biological effect of various physical mutagens **(Konzak *et al.*, 1975)**. Mutagens differ in their mechanism and mode of action in the biological system. Hence, the extent of reduction in growth is related to the mechanism of action for a given mutagen. The reduction in seedling height occurring with corresponding increase in dose of the mutagen suggested positive correlation with the doses. Such correlations have been established by earlier workers **(Ramani and Jodan, 1995; Odeigah *et al.*, 1998; Singh *et al.*, 1999; Kumar and Mishra, 1999); Larik *et al.*, (2009); Wani, (2007); Aparna *et al.*, (2013); Ramya *et al.*, 2014)**. Growth inhibition of seedling particularly at higher doses is mainly due to physiological injury in the seed and seedling. The factors, such as auxin synthesis production of diffusible growth retarding substance, changes in specific activity of enzymes and inhibition of DNA synthesis may be the other probable causes for the inhibition of growth following mutagenic treatments. Less injury may have resulted either due to mitotic delay or mutagenic arrest causing retardation of seedling growth **(Tripathi and Dubey, 1992)**.

5.1.3. Pollen sterility

The study of pollen sterility is another criterion of determining radio sensitivity. The data on pollen sterility as per cent of control are given in the **(Table 4.3)**. It is apparent from the experimental results that mutagenic treatments enhance pollen sterility in M_1 generation as compared to respective control. Pollen sterility has been found to increase with increase in doses. It clear from the data that pollen sterility increased with increase in the treatment of gamma rays suggesting the relationship with the dose dependent increase in the frequencies of meiotic chromosomal abnormalities. The increase in the sterility as a consequence of mutagenesis and mutagen induced pollen sterility has been reported in several crops including pulses **(John, 1996; Manapure and Patil, 1997; Odeigah *et al.*, 1998; Kumar and Mishra, 1999; Barshile *et al.*, 2006; Singh *et al.*, 2007; Aparna *et al.*, (2013)** and **Ramya *et al.*, 2014))**.

5.2. Studies in M_2 and M_3 generations

5.2.1. Frequency and spectrum of chlorophyll mutations

5.2.1.1. Frequency

The frequency of chlorophyll mutations per 1000 M_2 and M_3 plants, induced by gamma rays treatments are presented in **Table 4.4 and 4.5**. It appeared from the data that gamma rays produced greater frequency of chlorophyll mutation in Arkel cultivar of pea. Chlorophyll mutations were classified as per the classification of **Gustafsson, (1940)** and the same applied in the present study. Highest frequency (3.145 per cent) was depicted at 40kR dry and lowest frequency (0.673 per cent) was found at treatment for 05kR pre-soaked in M_2 generation. The highest frequency (1.913 per cent) was exhibited at 40kr dry and lowest frequency (0.326 per cent) was found at treatment for 10kR dry in M_3 generation.

As expected about 250-300 loci might be involved for breakdown of the chlorophyll apparatus in barley **Swaminathan, (1965)** and **Kharakwal, (2003)** in green gram reported involvement of considerable number of genes at different stage of plastid development as revealed from the plastid ultra structure of leaves. Hence, the probability of occurrence of such category of mutation is obvious with all mutagen treatments.

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effect of mutagenic treatments and have been reported in various pulse crops by several workers including **Gautam *et al.*, (1992); Singh *et al.*, (1999); Waghmare and Mehara, (2001); Barshile *et al.*, (2006); Singh *et al.*, (2007; Mahamune and Kothekar, (2012)**. In the present study, treatments differences in response to chlorophyll mutations were depicted as frequency of induced chlorophyll mutations.

5.2.1.2. Spectrum

The spectrum of mutations during segregating M_2 and M_3 generations for Arkel cultivar are presented in **Table 4.4 and 4.5**. Data reveals that in the present study, the chlorophyll mutations constituted mainly four kinds of mutations *viz*., *Albina, Chlorina, Xantha*, and *Viridis*. Out of the four chlorophyll mutants as recorded during the study, *Viridis* was found most frequency followed by *Chlorina, Xentha* and *Albina* respectively. These findings are in accordance with **(Kumar *et al*., 2007** and **Singh *et al*., 2007)**.

The occurrence of chlorophyll mutation after mutagenic treatments has been reported by **Singh and Yadav, (1991)**. Chlorophyll development seems to be controlled by the involvement of polygene in chlorophyll formation **(Gaul, 1964)** and the expressivity varies with the environmental effects **(Bahal and Gupta, 1982)**. In present study the spectrum of chlorophyll was not too wide as only four type's *viz*., *Viridis, Chlorina, Xantha* and *Albina* were exhibited. The similar finding have been reported by **Singh and Yadav, (1993)** and **Goverdhan and Lal, (2013)**. The conflicting reported on the frequency and spectrum of chlorophyll mutations in single cultivar may be due to different treatments between the cultivars used. Similar observations have been reported by different workers in legume **(Malik *et al*., 1999; Singh *et al.*, 1999; Makeen and Suresh, (2010)**.

5.2.2. Frequency and spectrum of viable mutations

5.2.2.1. Frequency

The data on frequency of viable mutations calculated on per 1000 M_2 and M_3 plants are presented in **Table 4.6 and 4.7**. The frequency of different types of viable macro mutations per 1000 M_2 and M_3 plants were scored at various development stages, particularly from flowering and maturity period. The presents study showed that all the doses of gamma rays induced viable mutations. It can be inferred from the finding that higher dose treatments may be less effective for induced mutation. The high frequency of macro mutation (4.878 per cent) was showed with the 40kR dry, while the lowest (1.017 per cent) frequency was depicted with the treatment of 05kR dry in M_2 generation. The earlier frequency of macro mutation (2.871 per cent) was found with the 40kR dry, whereas the lowest (0.330 per cent) frequency was recorded with the treatment of 05kR pre-soaked in M_3 generation. Many workers reported decrease in mutation frequency with the increase with in doses of mutagens **(Sharma, 1987** and **Kumar *et al.*, 2007)**. Such decrease in mutation frequency could attribute to saturation effect in a mutation sector involving multiple mutations **Gaul, (1961)**.

5.2.2.2. Total frequency

The data on total frequency of viable mutations (per cent) calculated on per 1000 M_2 and M_3 plants are presented in **Table 4.8** and **4.9**. Total mutated plants randomly increases with the increase in gamma rays doses with the highest frequency value under the treatment for 40kR dry (7.318 per cent) and lowest frequency being the under the treatment for 10kR pre-soaked (0.360 per cent) in M_2 generation.

The revealed data indicated that total mutated frequency randomly increases with the increase in gamma rays doses with the maximum value under the treatment for 40kR dry (4.280 per cent) and lowest frequency was found under the treatment in 20kR pre-soaked (0.351) **Table 4.9** in M_3 generation.

Gautam and Mittal (1998) also observed specificity of mutations to different mutagenic doses in und bean which may be attributed to the difference in the action of physical mutagen at molecular level. **Sinha and Bharti (1990)** in their study obtained mutant which was more vigorous and produced more large pods in size. Various types of morphological mutants were induced by different mutagenic treatment with respect to plant stature, maturity, pods and seed characteristic in M_2 and M_3 generation. Theoretically, each gene which has any agronomic importance can mute; hence, a wide spectrum of viable mutations can be expected in mutation experiments. As mention earlier, the probable cause of these macro mutations may be chromosomal aberrations, small deficiencies or duplication and most probably point or gene mutations, (**Kharkwal *et al.*, 2004)**.

5.2.3. Growth habit mutants

The dwarf, semi dwarf, tall early and late maturing, bold, long, and short mutants, brown, light brown and light green mutants, small, bold, wrinkled seeded mutants were isolated in M_2 and M_3 generations. The dwarf mutants were characterized by condensed nodes, shorter internodes and lower yield as compared to the control. The plants exceeding in height of respective control were placed in the tall category and the intermediate between the dwarf and the control were noted as semi dwarf mutants. The spreading mutants depicted branching parallel to the ground and such plants covered more area than the normal plants. Several workers have reported growth habit mutant in pea for example dwarf mutants **(Manapare *et al.*, 1998)**, semi dwarf **(Sharma *et al.*, 2005)**, tall mutants **(Hepziba and Subramanism, 2002)**.

5.2.3.1. Maturity mutants

Early maturing mutants were isolated M_2 and M_3 generation as compared to the normal period of the control. The early maturing mutants have been reported by **Gautam and Mittal,**

(1998). Synchronous maturing mutants have been obtained which were supported by the finding of **Singh, (1996)** and **Kharakwal *et al.*, (2004)**.

5.2.3.2. Pod mutants

These mutants were grouped into bold, long and short pods. Similar mutants have been reported by **Hepziba and Subramanian, (2002)**. The presences of short bold and long podded mutants were induced by different mutagenic treatments in M_2 and M_3 generations. Short podded mutant had relatively less number of seeds per pod with lower yield as compared to the control. Similar mutants in pea have been reported by **Gautam and Mittal, (1998)**. Bold and long poded mutants had higher number of seed per pod and increased grain yield over the parents are reported by **Gautam and Mittal, (1998)** and **Kumar *et al*., (2007)**.

5.2.3.3. Seed mutants

The mutants with varying seed morphology (*e.g*., bold, small and wrinkled) and seed testa colours (brown, light white, light green) were induced in M_2 and M_3 generations. The bold seeded mutants had increased 100 seed weight and seed yield as compared to the control. This is in accordance with the finding of **Mittal *et al*., (2001)**.

The mutants for varied testa colour such as brown and light white, light green have been induced in the M_2 and M_3 generations. Such mutants have been noticed in many pulse crops. For example, brown seed, light white and light green in pea by **Gautam and Mittal, (1998); Singh *et al*., (1999)**; **Kumar *et al*., (2007); Girija and Dhanavel, (2013)**. The simultaneous variation for yield and other morphological characters radiates a gross change, perhaps due to very closely linked group of genes. The gamma rays treatments induced a higher frequency resulting in higher mutation efficiency and an expansion of the spectrum of both chlorophyll and morphological mutations, thus offering more opportunities for selection.

5.2.4. Effectiveness and efficiency of mutagens in M_2 and M_3 generations

The usefulness of any pea in plant breeding depends not only on its effectiveness but also upon efficiency. **Konzak *et al.* (1965)** coined the terms ‘effectiveness’ and ‘efficiency’. Mutagenic effectiveness denoted the frequency of mutants induced by unit dose of a mutant (factor mutation/ doses) while mutagenic efficiency is a measure of the proportion of mutations in relation to undesirable changes (factor mutations/ sterility) like sterility, injury, survival etc. The lower efficiency of certain mutagens may be attributed to the use of low doses corresponding to their mutation induction **(Kharakwal, 2000)**. The higher efficiency of a mutagen indicates relatively less biological damage (plant survival, sterility etc.) in relations to mutations induced **(Table 4.10-4.11)**. The efficiency of mutagenic agents not only depends on the biological system but also on physiological damage, chromosomal aberrations and sterility

induced due to mutation. The efficiency of any mutagen would therefore depend on its effectiveness and efficiency. The effectiveness of a mutagen is of theoretical importance but does not have any immediate practical significance **(Tan *et al.*, (1990)**; **Vinod and Sharma, (1998); Gaul and Astveli, (1996); Waghmare and Mehra, (2001); Sharma *et al.*, (2005); Kavithamani *et al.*, 2008; Pavadai *et al.*, (2010); Bhosle and Kothekar, (2010); Bharathi *et al.*, (2014)**. For practical purpose, one should desire to obtained high efficiency with an optimum dose of mutagen.

The experimental finding indicated that mutation frequency (chlorophyll as well as macro mutations) showed dose dependence, the maximum increase occur at lower doses of gamma rays. Mutation frequency increased in the gamma treatments in comparison to individuals' mutagenic treatments. This is well supported by **Kharakwal *et al.* (2004)** who observed that mutagenic effectiveness and efficiency were highest at lower doses as observed on lethality and seed sterility in M_1 generation and chlorophyll mutants in M_2 and M_3 generations. The lower doses of different mutagens were found most effective and efficient. The finding were well strengthened with the finding of **Sharma *et al.*, (2005)** who stated that the effectiveness was the highest at the 100Gy and it was lowest 400Gy indicating that the proportionate increase in mutation rate was much lower than the proportionate increase in dose of mutagen. The efficiency was higher at the lower dose of the mutagens, which decreased further with the increasing dose of mutagens, further indicating the proportionate increase in the mutation rate was much less than the proportionate increase in biological damage. At higher doses of mutagens there was no further decrease in the mutagens efficiency and the value appeared to have become static.

5.3. Induced variability for quantitative characters (micro mutations) in M_2 and M_3 generations

5.3.1. Analysis of variance

The analysis of variance for all the characters studied in M_2 and M_3 generations of pea cultivar Arkel are presented in **Table 4.12** and **4.13** respectively. While most of the characters in all the generations showed significant difference among the treatments under study. Mutations affecting quantitative characters can best inferred by the estimate of mean performance and coefficient of variation and other genetic parameters in the mutagen treated population. A polygenic mutation of micro mutation is a mutational event which causes only small modification in phenotype of a trait. Such mutation should be useful for improving quantitatively inherited traits (*viz*., seed yield per plants) without disturbing the major part of the genotypic and (or) phenotypic architecture of the crop plants. Many workers have

demonstrated the role of micro mutation in crop improvement **(Gaul, 1970** and **Sharma *et al*., 2005)**.

5.3.2. Mean performance

The estimates increase in mean values in M_2 and M_3 generations over controls could be attributed to elimination of lethal gene (s) after selfing or elimination of undesirable morphological mutants and in fertile plants **(Borojevic, 1965)** or higher magnitude of induced individual changes in positive direction **(Gregory, 1965)**. The experimental finding clearly showed significant difference in the mean values of various quantitative traits in the traits in the treated population with their respective controls. The mean of different traits shifted either to positive or negative direction away from the control due to mutagenic treatments. Similar finding were observed by the many workers like **Ahmad and John, (1996); Singh *et al*., (2000)**. The occurrence of polygenic mutations with unequal frequencies of plus and minus effects may be considered as a cause of shift in mean in positive and negative directions. Negative shift in mean may be due to the occurrence of deleterious or harmful mutations whose frequency was more than mutation of desirable nature.

In the present study **Table 4.14-4.15** many treatments showed slight increase or decrease in the mean values of different characters. In other words, they were comparable to the means of the control this could be due to occurrence of polygenic mutations with plus and minus effects unequally distributed. Under such conditions, only the variability is enlarged without changes in the mean. **Gaul and Astveli (1966)** proposed that mean of the M_2 generation did not shift away from the control due random mutations in polygenes. The mean performance of mutagen treated population remaining unchanged indicated bi-directional mutational mutations. Besides, the mean performance of a population having equal proportion of favourable and unfavourable genes would remain unchanged, since mutations in plus and minus direction will be equally likely.

The mean performance of quantitative characters showed improvement in most of the mutagenic treatments in M_2 and M_3 generations. Some mutagenic treatments induced higher significant positive shift in mean over respective controls for certain characters in M_2 and M_3 generations for example seed yield per plants (g) was 22.80g in M_2 and 23.83g in M_3 generations at on 20kR dry dose. The selection of promising plants with sufficient seeds was advance in M_3 generation. It is possible that the selection pressure tended to move from extremely low performing lines in favour of relatively higher performing lines and thus producing increased mean in M_3 generation in comparison of M_2 generation. There are several reports on significant reductions in yield and its components in treated populations of pea,

Sinha and Bharti, (1990) and **Khan *et al*., (1989); Murugan *et al*., (1995)** in cowpea. The low mean value may be due to radiation induced sterility, leading to significant reduction in mean values. **Bhatia and Swaminathan, (1962)** have reported such declination in mean value as the result of frequent occurrence of mutation with detrimental effect.

5.3.2a. Mean performance of moisture stress condition

Among the treated population, a number of mutants with moisture stress conditions in one or more traits (morphological, physiological and yield parameters) were isolated from the cultivar Arkel of pea in M_2 and M_3 generations **Table 4.16-4.19**. Global climate change in the form of increasing temperature and fluctuating soil moisture conditions including drought, is researched to decrease the yield of food crops over the next fifty years **(Leakey *et al.*, 2006)**. Drought stress after flowering is one of the most common and serious environmental limitations to yield in pearl millet, resulting in 50 per cent flowering loss **(Mahalakshmi *et al.*, 1987)**. Drought is the most damaging abiotic stress to soybean production, and in the USA, dry land soybeans yield approximately 60-70 per cent less than irrigated system **(Egli, 2008)**. The adverse effect of drought on plant structure and function such as xylem embolism, reduced carbohydrate pool size, leaf and fine root production on the ability of plants to resist pathogen attacks, the impacts on soil microbial dynamics, decomposition and nutrient supply processes and shifting competitive abilities between plant species could not be under estimated **(Ciais *et al.*, 2005)**. Physiological traits, such as osmotic adjustment, contents of ABA, chlorophyll, proline and soluble sugars and toxic removal mechanisms such as peroxidise or superoxide dismutase activity etc., contribute to dehydration tolerance **(Luo, 2010)**. Plant developmental traits such as early vigour or phonology may be particularly significant in water limiting conditions **(Cairns *et al.*, 2009)**. Seed size and early seedling vigour were found to be associated with drought tolerance in pearl millet **(Manga and Yadav, 1995)**, wheat **(Robetzke and Richards, 1999)**, sorghum **(Harris, 1996)** and rice **(Cui *et al.*, 2008)**. Genotypic differences that exist for relative water content under drought were well documented in soybean **(James *et al.*, 2008)** and other crops. A positive relationship was observed between grain yield and relative water content measured during the reproductive stage in pea, where the high yield selections maintained significantly higher relative water content than the low yield selections **(Tahara *et al.*, 1990)**. When leaf relative water content falls to around 70 per cent photosynthesis in most species becomes irreversibly depressed **(Lawlor and Cornic, 2002)**, and thus the resistance of the photosynthesis apparatus to desiccation is also a potential trigger for stomata closure. The measurement of ion leakage and further estimation of membrane stability had been used as criteria for selection for drought resistance in wheat **(Blum and**

Ebercon, 1981) and rice **(Tripathy *et al.*, 2000)**. A positive association between cell membrane stability and high phospholipids content was observed in drought tolerant maize cultivars **(Premachandra *et al.*, 1991)**. However, the ion leakage method is time consuming and needs a lot of replication to capture genotypic differences. Measurement of chlorophyll fluorescence was used as a non destructive measure of drought avoidance in wheat **(Araus *et al.*, 1998)** and maize **(Earl and Davis, 2003)**. An extensive amount of information is available on the value of root traits in relation to drought avoidance in crops **(Coutois *et al.*, 2009; Hochholdinger and Tuberosa, 2009; Hodge, 2009; Maurel *et al.*, 2010; Yamaguchi and Sharp, 2010)**. Estimation of leaf relative water content is quite simple and certainly to a large number of plants. It has been suggested that plant water status, rather than plant function, controls crop performance under drought. Therefore, those treatments that can maintain higher LWP and RWC are drought resistant simply because of their superior internal water status **(Kamoshita *et al.*, 2008)**. A positive association between cell membrane stability phospholipids content was observed in drought tolerant maize cultivars **(Premachandra *et al.*, 1991)**.

5.3.3. Variability performance

Mutagenic treatments are known to enhance variability in quantitative characters. Experimental results of the mutagenically induced variability measured as coefficient of variation, increased in the treated population as compared to the respective controls for all the polygenic characters in general. It has been found that the genetic variability increased with the increased in mutagen doses. The lack of linearity may be due to the elimination of mutations through gametic or zygotic selection, which eliminates a large number of new genotypes from the population soon after mutagenic treatments and thus reduces the potential genetic variability. The high doses of mutagens inducing greater variability during the present study have also be reported in many other pulse crops (**Manapure and Patil, (1997); Singh *et al.*, (2000); Kharkwal *et al.*, (2004)**. There are many reports, which suggest that genetic variability increases by physical treatments, (**Ignacimuthu and Babu, (1992); Vanaiarajan and Das, (1997); Khan and Wani, 2006; Singh *et al.*, 2006; and Khan and Goyal, 2009)**.

As in the present study, the differences in the intensity of variation in different plants attributes at the same dose level were also reported by **Vanniarajan** and **Das, (1997)**. This might be explained on the basis that the different characters of the plants are governed by different genes or set of genes, which lead to differential response of the mutagenic treatments. The magnitude of variability was generally lower in all the mutagenic treatments in M_3 generation than the corresponding treatments in M_2 generation with few exceptions. The

decline of polygenic variability in M_3 generation as compared to M_2 generation has been reported by **Murugan** and **Subramaniam, (1993)**.

The increase in variability in M_2 generation as compared to M_3 generation may be attributed to the phenomenon of segregation. It was postulated that genetic variability remains in hidden condition in the heterozygous state in M_2 generation but exposed in later generation due to homozygous at different loci **(Scossiralli, 1968)**.

5.4. Studies on genetic parameters in M_2 and M_3 generations

Induced genetic variability in M_2 and M_3 generations of pea *cv.* Arkel was assessed with the help of different genetic parameters. The data on this parameter are depicted in **(Table 4.20** and **4.21)**.

5.4.1. Coefficient of variation in M_2 generation

The genetic variability is one of the prerequisites for crop improvement. The values of genotypic coefficient of variation (GCV) for different characters ranged from 1.56 (days to maturity) to 12.81 (number of primary branches per plant) **(Table 4.20)**. The higher values of genotypic coefficient of variation was noticed for number of primary branches per plant, plant stand per plot, number of pods per plant, number of seeds per pods, seed yield per plants, biological yield per plant, number of secondary branches per plant, pod length, 100 seed weight, plant height, harvest index, days to 50 per cent flowering and days to maturity.

Selection for improvement of such traits showing higher variability may be practiced in the subsequent generations. The observation corroborate with the earlier findings of **Igancimuthu and Babu, (1992); Ahmad and Yaqoob, (1993)** and **Hipziba and Subramaniam, (2002)**. In general, biometrical analysis has shown increase in phenotypic variation in generations following gamma rays treatments, particularly in self pollinated crop like pea. Phenotypic variation is mainly due to mutations of the genetic factors influencing the quantitative characters. From the plant breeding point of view this is an important effect because large genetic variation mean the possibility of increased chances of selection and higher chances for improvement. Phenotypic coefficient of variation was higher than the corresponding genotypic coefficient of variation. Higher values of phenotypic coefficient of variation was found for number of primary branches per plant, plants stand per plot, number of pods per plant, number of seeds per pods, seed yield per plants, biological yield per plant, number of secondary branches per plant, pod length and 100 seed weight, remaining characters showed low to moderate PCV. This is in close agreement with the earlier reported of **Jain *et al.*, (1995); Sharma *et al.*, (1996); Singh *et al.*, (2001); Singh *et al.*, (2002); and Momin and Mishra, (2004).**

5.4.2. Heritability (broad sense) in M_2 generation

The estimated heritability (in broad sense) ranged from 57.05 (Plant height) to 77.82 (number of seeds per pod) **(Table 4.20)**. High heritability values was exhibited for most of the quantitative traits like number of seeds per pod, number of pod per plant, number of secondary branches per plant, pod length, biological yield per plant, days to 50 per cent flowering, plant stand per plot, seed yield per plant, number of primary branches per plant, 100 seed weight, days to maturity, harvest index and plant height while rest of the quantitative traits showed moderate heritability values. These results indicate that a major part of phenotypic variability might be attributed to utilizable genetic cause through induction of mutations. These observations collaborate with the finding of **Pathirana, (1990); Jain *et al.*, (1995); Singh *et al.*, (2001); Hipziba and Subramaniam, (2002); Singh *et al.*, (2002); and Momin and Mishra, (2004)** in pulses crops. **Byregowda *et al.* (1997)** reported high heritability estimates for seed yield per plant and pods per plant. The selection for improvement of characters possessing high heritability would be useful.

5.4.3. Genetic advance in M_2 generation

A high genetic advance expressed as per cent of mean was recorded in **Table 4.20**, for plant stand per plot, plant height, biological yield, days to maturity, number of pods per plants, harvest index, days to 50 per cent flowering, seed yield per plants, 100 seed weight, number of primary branches per plant, number of seeds per pod, pod length and number of secondary branches per plant. However genetic advance were found low for number of secondary branches per plant and pod length. Genetic advance as percent of mean was found highest for number of secondary branches per plant, plant stand per plot, number of pods per plant, number of seeds per pod, seed yield per plants; moderate for number of secondary branches per plant, biological yield, pod length, 100 seed weight, plant height and lowest values for harvest index and days to maturity.

The traits which showed high genetic advance are governed by additive genes, hence they potentiality for improvement at five per cent selection intensity. Similar results were reported by **Ram and Singh, (1993); Sood and Garten, (1994); Singh *et al.*, (2002);** and **Momin** and **Mishra, (2004)**.

5.4.4. Coefficient of variation in M_3 generation

In any crop improvement programme evaluation of genotype for identification of genetic variability in various traits is very important, mostly when considering the adoption of new traits (like some physiological traits in present study) to create further variability through gamma radiations is of vital importance. Therefore, for successful and efficient crop

improvement programme it would be desirable to have systematic and detailed information on yield component characters. The magnitude **(Table 4.21)** of genotypic coefficient of variance (GCV) was higher for plant stand per plot, number of seeds per plant, number of primary branches per plant, number of pods per plant, biological yield, seed yield per plant, pod length, number of secondary branches per plant, 100 seed weight, plant height, harvest index and days to maturity. Phenotypic coefficient of variation (PCV) was found higher than the corresponding genotypic coefficient of variation except for all characters higher PCV was found for plant stand per plot and number of primary branches per plant. Similar results were reported by **Naidu *et al.*, (1991); Byregowda *et al.*, (1997); Singh *et al*., (2001); Hipziba and Subramanian, (2002); Singh *et al.*, (2002)** and **Momin** and **Mishra, (2004)**.

5.4.5. Heritability in M_3 generation

Heritability specifies the proportion of the total variability that is due genetic cause. It is a good index of the transmission of characters from parent to the offspring and helps in determining whether phenotypic differences observed among individuals are due to genetic or environment factors. Therefore, the characters with high heritability values are of much importance to plant breeder than those, which are less heritability and more susceptible to environmental fluctuations. Low heritability indicates that the character is highly influenced by the environmental fluctuation and one has to raise large population for selecting the desirable genotypes. Heritability estimates revealed that low heritability was observed for plant height, days to maturity, harvest index, number of primary branches per plant, days to 50% flowering, 100 seed weight, number of secondary branches per plant, plant stand per plot, seed yield per plant, biological yield, number of seeds per pod, number of pods per plant and pod length. Similar results were reported by **Ram and Singh, (1993); Sood and Garten, (1994); Singh *et al.*, (2002)** and **Momin and Mishra, (2004)**. **Johnson *et al.* (1955)** suggested that heritability estimates along with genetic advance shall be more helpful in predicting gain under phenotypic selection than heritability estimates alone. These results, indicating greater scope for successful selection as these traits might be governed by the additive gene action and improvement with respects of these characters could be brought about by phenotypic level.

5.4.6. Genetic advance in M_3 generation

Genetic advance is directly related with the heritability as it gives an idea about the expected genetic changes on account of selection applied to a particular trait. The earlier genetic advance expressed as per cent of mean was recorded for plant stand per plot, biological yield per plant, plant height, harvest index, number of pods per plant, seed yield per plant, 100 seed weight, days to 50 per cent flowering, days to maturity, number of seeds per pod, pod

length, number of primary branches per plant and number of secondary branches per plant. Genetic advance as percent of mean was found highest for plant stand per plot, number of seeds per plant, number of pod per plant, number of primary branches per plant, biological yield, pod length, seed yield per plant and lowest values for number of secondary branches per plant, 100 seed weight, plant height, harvest index, days to 50 per cent flowering and days to maturity.

In the present study, high heritability coupled with high genetic advance was observed for pod length and plant stand per plot. Hence selection would be effective for further improvement of these traits. **Johnson *et al.* (1955)** have suggested that heritability estimates along with genetic advance shall be more helpful in predicting gain under phenotypic selection than heritability estimate alone. This trend suggested that further selection can be done for improvement of these traits. These traits are more likely to be controlled by additive genetic component hence they can be fixed.

5.5. Character association studies in M_2 generations

Correlation coefficient gives an idea about the various associations existing with yield and yield components. Phenotypic correlation determines the association between two variables which can be directly observed. It includes both genotypic and environmental effects and therefore differs under different environmental conditions. On the other hand, the inheritance association may be either due to pleiotropic action of genes or due to linkage or more likely both.

Breeding for high yield is the major objective in any crop improvement programme. A direct selection for yield is often misleading because yield itself has number genes as it is a dependent character on constellation of yield component characters highly influenced by environmental factors. As efficiency of selection mainly dependent up on the knowledge of association of characters with yield, therefore to bring a change in yield or other yield related traits to a desired level, proper understanding of association between yield, yield component traits or new traits which are being introduced in breeding programme is must. This approach might be useful in selection of traits association with the highest expression of yield and improvement of the characters without sacrificing much in other traits. In general genotypic correlation coefficients were higher in magnitude than the phenotypic correlations. This indicates that there was high genetic relationship between the traits under study and environmental has not much influencing in reducing their actual association.

The data illustrated in **(Table 4.22)** indicated that seed yield per plant showed significant high positive genotypic correlation for biological yield, number of pod per plant, number of seeds per pod, plant stand per plot, number of primary branches per plant, number of

secondary branches per plant, pod length, days to 50 per cent flowering, 100 seed weight, plant height, days to maturity, while lowest value was recorded for harvest index. Estimate of phenotypic correlation characters studied are presented in (**Table 4.23)** to revealed that seed yield per plant showed significant high positive correlation for number of pod per plant, biological yield, plant stand per plot, number of seeds per pod, number of primary branches per plant, pod length, days to 50 per cent flowering, number of secondary branches per plant, 100 seed weight, harvest index, plant height, while lowest value was recorded for days to maturity. This positive relationship between components reflects promise of improvement in yield in new genotypes as a result of appropriate nicking of the component genes of desired value. The characters, seed yield per plant, number of pods per plants, biological yield per plant and number of seeds per plant was identified yield contributing characters which are in agreement with the present study. **Mehra *et al.* (1994)** derived the information on correlation from data on seven yield related traits in the M_2 and M_3 generations of gamma rays irradiated soybean varieties. In addition, these traits are important heritable components of yield and should be given top priority for making selection of further improvement seed yield in the selected mutant lines of pea. These results are in comparison with **Kumar *et al.*, (1999); Tyagi and Srivastava, (2002); Chaudhary and Sharma, (2003); Ranga *et al.*, (2006); Patel *et al.*, (2006)**.

5.6. Correlation coefficient studies in M_3 generations

Information on the nature and extent of correlation between yield and its component is helpful for the choice of characters in the selection programme aimed at improvement of yield. Correlation studies provide better understanding of yield components, which help the plant breeders during selection **(Johnson *et al.*, 1955)**.

The present study (**Table 4.24)** indicated significant positive correlation for biological yield per plant, number of primary branches per plant, number of seeds per pod, number of secondary branches per plant, number of pods per plant, pod length, plant stand per plot, 100 seed weight, plant height and days to maturity, while lowest value was found for days to 50 per cent flowering. The harvest index had negative correlation with yield indicated that there was genetically strong association of the traits with seed yield per plant. The phenotypic correlation characters studied are presented in (**Table 4.25)** the study seed yield per plant showed significant positive correlation for biological yield per plant, number of pods per plant, number of primary per plant, number of seeds per pod, number of secondary branches per plant, plant stand per plot, pod length, 100 seed weight, plant height, while lowest value was recorded for days to 50 per cent flowering. The days to maturity and harvest index had negative correlation

with seed yield per plant. This suggested that top priority should be given to these traits while making selection for improvement of yield in the selected mutant lines of pea. Seed yield per plant also showed highly significant and positive correlation with biological yield, number of pod per plant, number of primary branches per plant and number of seeds per pod **Mehra *et al.* (1994)** derived the information on correlation from data on seven yield related traits in the M_2 and M_3 generations of gamma rays irradiate soybean varieties.

Experiment results indicated that values of genotypic correlation were higher than the phenotypic correlation for most of the character pairs of the mutants. This is possible due to masking or modifying effects of environment on character pair at genetic level **(Johnson *et al.*, 1955)**.

If any character, having low heritability shows high correlation with the valuable or economical character. This information may give misleading results of selection. Under such condition a character having high heritability and high correlation with the economic trait it's chosen to make selection through component character with heritability. In addition, these traits are important heritable components of yield and should be given top priority for making selection of further improve seed yield in the selected mutant lines of pea. These results are in comparison with **Chaudhary and Sharma, (2003); Ranga *et al.*, (2006); Patel *et al.*, (2006)**.

5.7. Path correlation analysis in M_2 generation

The path coefficient analysis provides an effective means of partitioning the correlation coefficients into direct and indirect effect of the component characters. Selection on the basis of direct and indirect effects is much more useful. Path analysis reveals whether the association of different quantitative characters with the yield is due to their direct effect of yield or is a consequence of their indirect effect via component characters.

The present investigation based on analysis of direct and indirect effect of yield component had brought to light that days to 50 per cent flowering, number of primary braches per plant, number of secondary branches per plant, number of seeds per pod, number of pods per plant, pod length, plant height, days to maturity, plant stand per plot, 100 seed weight, biological yield per plant, harvest index and seed yield per plant at phenotypic and genotypic levels in M_2 generation.

This suggests true relationship between these traits with seed yield per plant and direct selection for these traits will be rewarding for yield improvement. In the present study, **(Table 4.26 – 4.27)** revealed that genotypic analysis had high direct effect was found from number of pods per plant, number of seeds per pod, plant stand per plot, number of secondary branches per plant, plant height towards in seed yield; however, it's negative direct effect was for

observed pod length, days to maturity, days to 50 per cent flowering, harvest index, biological yield per plant, number of primary branches per plant and 100 seed weight towards in seed yield. Similar results were observed by **Kumar *et al.*, (1995); Kumar *et al.*, (1999); Ranga *et al.*, (2006); Patel *et al.*, (2006)**. The knowledge of such relationship has direct bearing selection, and if this knowledge is applied appropriately to the selection, the gain per selection cycle may be much higher as compared to selection to new genotypic randomly. It may help not only in picking up new desirable genotype, but can reduce the selection pressure. However, it's dependence for selection with the changed in material and environment. Hence, selection should not entirely be based such knowledge until and unless the relationship is confirmed.

5.8. Path analysis in M_3 generation

The correlation coefficient simply indicates the degree of association among the characters contributing towards economic yield and its knowledge with regard to seed yield and seed yield components could be helpful in selection. However, it does not provide measure of causal relationship existing among variables. Therefore, it becomes essential to identify components of yield and their relative contribution. The path coefficient analysis helps in understanding the causal factor better because it divides the total correlation of paired traits into direct and indirect effect via other characters.

Path analysis differs from simple correlations in the sense that it points out the causes and their relative importance, whereas the correlations simply measure the mutual association.

In the present investigation path coefficient analysis was carried out for taking seed yield per plant as the dependent variable and remaining twelve traits are independent variables. The present investigation **(Table 4.28- 4.29)**, the positive direct effect on seed yield per plant (dependent variable) was the function of (independent variable) number of seeds per pod, number of pods per plant, harvest index, biological yield per plant, plant stand per plot, 100 seed weight; while it's high negative direct effect was recorded for pod length, number of secondary per plant, days to 50 per cent flowering, days to maturity, plant height, number of primary branches per plant toward in seed yield per plant. **Mishra and Pradan (2006)** reported that pods per plant and 100 seed weight appeared to be major yield contributing traits. Similar results were observed by **Kumar *et al.*, (1995)** and **Kumar *et al.* (1999).**

5.9. Mutagenic effect on seed quality parameters in M_1 mutant seeds

In the present study several methods were employed to measure the seed quality parameters such as percent seed viability, percent seed germination, speed of germination, seed vigour index and vigour index mass on storability and seed quality of pea seeds under tri-monthly storage period in the 24 months. Viability and vigour of the seed varied from source to

source as the locality factors influenced the storability of seed. The seeds from different sources possess different quality values, physical structures. These factors determine the longevity of seed in the storage.

5.9.1. Analysis of variance

The treatments *i.e.* mean sum square due to gamma rays treated **(Table 4.30 to 4.33)** showed significant differentiate to all four seed quality traits *viz.* germination per cent, speed of germination, seed vigour index and vigour index mass except germination percent in 15th month of gunny bag and seed vigour index in 18 month of gunny bag was recorded non significant.

5.9.2. Per cent seed viability

In the present investigation **Table 4.34**, indicated that maximum per cent seed viability under dry and pre-soaked with controls before storage. After 24 months of storage, minimum percent seed viability loss was recorded under higher doses of gamma rays under 40kR dry. Difference of seed viability loss observed only 31.50 per cent followed by 28.50 per cent in 35kR dry, 25 per cent in 30kR dry, 24 per cent in 25kR dry, and 24.35 per cent in 20kR dry. The maximum difference of viability loss was observed under wet and dry control (29.00 per cent).

Table 4.34 showed that in all the treatments stored in different containers (gunny and plastic bags) the maximum seed germination was found in all the treatments stored in plastic bags. The per cent seed viability decreases with increase in store after period in different treatments of pea. **Nozzolillo and Lorenzetti (1998)** also reported that viability of pea seeds decreases more rapidly as compared to common cultivars, during storage period.

5.9.3. Per cent seed germination

With the advance in the storage period, irrespective of seed source all the seed quality parameters were gradually decreased **(Table 4.35 -4.36)**. It is generally seen that reduction in germinability depends on duration of aging. Germination decreases with increase in ageing period, as seen by **Dharmalingam (1995)** in maize due to natural aging. In stored seeds, aging is a universal physiological phenomenon followed by deterioration resulting in the loss of viability. Usually it progresses at a faster rate under stress or unfavourable conditions. The mechanism of deterioration which is the final stage of aging process is still an enigma. Generally, seeds stored in moisture impervious sealed containers store better compared to moisture pervious containers under ambient storage conditions. The prevailing relative humidity and temperature of atmosphere influence greatly the longevity of the seeds, since

moisture content of the seeds fluctuates more in the moisture pervious containers than in the moisture vapour proof containers.

The seeds stored in plastic bags (700 gauge) recorded significantly higher per cent seed germination as compared to the seeds stored in gunny bag at 3, 6, 9, 12, 15, 18, 21, and 24 months of storage. This indicated the slower deterioration of seed stored in the plastic bag compared to the seeds of gunny bag. Further, it was also observed that there was no variation in moisture content of seeds stored in plastic bag, which means the moisture content of seeds remained constant throughout storage period. But the moisture content of seeds stored in gunny bag exhibited a lot of fluctuation and as also higher compared to the seeds stored in plastic bag throughout the storage period. Similar results were observed by **Kurdikeri, (1991)**.

The seeds stored in plastic bag recorded significantly higher seed germination per cent under the treatment in 20kR dry (69.50 per cent) followed by 10kR dry (68.25 per cent), while lowest seed germination per cent was found in 40kR dry (60.25 per cent) as compared to the seeds stored in gunny bag at the end of 24 months of storage. A significantly higher seed quality parameters in the seeds stored in polythene bag may be due to higher seed germination per cent of seeds with constant seed moisture maintenance. Such results are reported by **Tammanagouda, (2002)** and **Arati, (2000)** in pulses. Seeds lose their viability and vigour very fast under ambient conditions as the changes in environmental conditions like temperature and humidity. So storing of pea seeds in plastic bags maintained viability and vigour at higher irradiated treatments order (above minimum seed certification standard) of compared to storing them in gunny bag.

5.9.4. Speed of germination

The speed of germination of pea seeds at bimonthly intervals was recorded in 3, 6, 9, 12, 15, 18, 21, and 24 months of storage of containers *viz.*, gunny and plastic bag **(Table 4.37-4.38)**. At the end of the 24 months storage period, the seed stored in a plastic bag (700 gauge) recorded high speed of germination in the treatment for 20kR dry (29.70), followed by 15kR dry (29.60), while the cloth bag recorded the lowest speed of germination in the treatment for 40kR dry (24.85). This indicated the slower deterioration of seed stored in the plastic bag compared to the seeds of gunny bag. Faster initiation of activity within the seeds due to the imbibed water may have indicated the speed of germination. This was supported by **Kalajbondi *et al.* (2003)** who reported that soaked seeds had germination and shoot length in kagzil, lime however not much variation was marked **Maguire, (1962)** reported high value of speed of germination indicate high seed vigour.

5.9.5. Seed vigour index

Seed vigour also followed a trend similar to seed germination per cent, *i.e*., it increased with the storage duration up to 24 months and then declined before the fall in seed germination **Table 4.39- 4.40**. Comparatively higher seed vigour was recorded in treatment 20kR dry (962.25) gunny, 15kR dry (1013.10) plastic followed by dry control (917.84) gunny, 20kR dry (917.84) gunny, 20kR dry (1009.81) plastic bag, whereas the after 24th month of storage lowest seed vigour index in both containers was showed 40kR dry (591.97 and 614.07) radiated treatment. A significantly higher seed quality parameters in the seeds stored in plastic bag may be due to higher seed vigour index with constant seed moisture maintenance. Such results are reported by **Tammanagouda (2002)** and **Arati, (2000)** in pulses.

5.9.6. Vigour index mass

Seed packing materials shows significant influence on vigour index mass at the end of 3-24 month storage periods. The data obtained **(Table 4.41-4.42)** at the end of 24 month storage period showed that highest vigour index mass in gunny and plastic bag in the treatments 05kR dry (2.27) gunny, 20kR dry (2.61) plastic bag followed 20kR dry (2.26) gunny, 05kR dry (2.43) plastic bag and 10kR dry (2.20 and 2.36) respectively. While the 24 months of storage lowest vigour index mass in gunny and plastic bag were found in 40kR dry (1.55 and 1.84) radiated treatments. This indicated the slower deterioration of seed stored in the plastic bag as compared to the seeds of gunny bag. Such results were reported by **Tammanagouda (2002)** and **Arati, (2000)** in pulses. So storing of pea seeds in plastic bags maintained viability and vigour at higher irradiated treatments in order (above minimum seed certification standard) of compared to storing them in gunny bag. Vigour is defined by the ISTA as "the sum totals of these properties of the seed which determination the potential level of activity and performance of a dormant seed **Perry (1972)**.