INDUCTION OF LEAF SPOT RESISTANCE AND IMPROVEMENT IN QUALITY OF AMARANTH THROUGH MUTATION

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Induction of leaf spot resistance and improvement in quality of amaranth through mutation"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled " **Induction of leaf spot resistance and improvement in quality of amaranth through mutation** " is a record of work done independently by **Kum. Binu Venuganan Panickar.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Binu Venuganan Panickar

Dedicated to My Family

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Introduction

INTRODUCTION

Amaranthus is a major leafy vegetable having great potential for combating under and malnutrition in dietary. It is the most important popular leafy vegetable of Kerala. It occupies a unique place due to its easiness to culture, fast growth rate, high response to added fertilizers and organic manure and adaptability to varying agroclimatic conditions (Grubben and Van Sloten, 1981). Because of its low production cost and high productivity, amaranth is considered to be the cheapest leafy vegetable in the market and could be rightly described as a "poor man's vegetable".

Amaranth, a multipurpose plant is grown for vegetable, grain and for ornamental purposes. Conventionally, five species namely, *Amaranthus tricolor, A. spinosus, A. dubius, A. viridis,* and *A. blitum* are considered as vegetable types. *A. tricolor* is the principal vegetable species consumed worldwide, particularly in the South East Asian countries (Prakash and Pal, 1991). *A. hypochondriacus, A. caudatus* and *A. cruentus* are the important grain species. Every 100 g of edible portion of *Amaranthus tricolor* contain 4 g protein, 2.7 g minerals, 397 mg calcium, 349 mg iron, 341 mg potassium, 9200 I.U. vitamin A, 99 mg vitamin C, 247 mg magnesium, 83 mg phosphorus, 230 mg sodium, 0.03 mg thiamin, 88 mg chlorine, 1.2 mg niacin and 0.3 mg riboflavin (Gopalan *et al.*, 1989). Seeds of *Amaranthus hypochondriacus* contains essential aminoacids such as lysine (5.5 g/16 g of N), methionine (2.6 g/16 g of N) and are rich in protein (15.6%) (NRC, 1984). The main constraint to their nutritional exploitation is the presence of some anti-nutritional factors like oxalates and nitrates in the leaves (Gupta and Wagle, 1988).

Apart from its nutritive value, absence of any serious pest or disease in the past has made it a popular leafy vegetable cultivated throughout India. However, a ravaging leaf spot disease, has been recently observed in the crop in Kerala (Kamala *et al.*, 1996). The causal organisms of leaf spot disease are *Rhizoctonia solani* Kuhn and *Colletotrichum capsici* (Syd.) Butter and Bisby. *A. tricolor* is infected by *Rhizoctonia solani* (Gokulapalan *et al.*, 1999 and Krishnakumary, 2000). Incidence of leaf spot disease is very severe in Kerala during rainy season causing considerable economic loss owing to reduction in marketability of the produce. Even total loss in yield was observed when plants were infected in the seedling stage. The effective way to combat the disease is the use of disease resistant varieties. Varieties of amaranth differ in disease resistance. In Kerala, all the *A. tricolor* cultivars including the commonly cultivated red varieties Kanara Local and Arun are highly susceptible to the disease whereas Co.1 (*A.dubius*) and a few grain types (*A. hypochondriacus*) were found resistant to the disease (Krishnakumary, 2000). The conventional plant protection measures for the control of disease are inefficient and undesirable from the point of view of human health and environmental hazards.

Many scientists have reported that radiation is as efficient as hybridization in supplementing genetic variability for selection. Induced mutations are the ultimate source of variability for having a positive selection response in crop plants. The main advantage of mutation induction is the ability to change one or a few characters of an otherwise outstanding cultivar without altering the remaining genotype. It is well known that a crop plant can be improved in productivity, resistance to various stresses and adaptation to environment, when genetic variability for that particular trait is available in the considered population or species. Under these circumstances, mutation breeding will be an efficient and alternate method of breeding in amaranth. In Amaranth, very little work has been done so far. Hence the present investigation on mutation breeding was taken up as a preliminary trial in the broad area of 'Induction of leaf spot resistance and improvement in quality of Amaranth through mutation'. The objectives are the following:

1) To standardize the optimum dose of the physical mutagen (Gamma ray) for inducing variability in *Amaranthus* spp.,
2) To induce leaf spot resistance and
3) To study the differential response of the mutagen on morphological, biometrical and quality attributes in M_1 and M_2 generations.

Review of Literature

2. REVIEW OF LITERATURE

Induced mutagenesis has now become one of the accepted tools to improve the economic traits of the crop plants. It is being used to a larger extent in genetic research, both in creating variability and in developing improved cultivars (Micke, 1975). In conventional plant breeding, variation is generated by hybridization and selections are made from the resulting segregating generations. Induced mutagenesis can either supplement hybridization or replace it as a source of variability.

Ionizing radiations provide a handy tool to enhance the natural mutation rate thereby enlarging the genetic variabilities and increasing the scope for obtaining the desired selections. Gamma rays have been extensively used by several workers to induce genetic variabilities in several crops. The term mutation was derived from the Latin word 'mutars' to denote change. Mutagenic actions of X rays was discovered by Muller in 1927 on Drosophila and in 1929 by Stadler in barley and maize. Gustafsson (1947) recognized the practical utilization of radiation to induce useful mutants. Sigurbjornsson (1969) in a detailed analysis of the specific role of induced mutations have clearly projected out mutation breeding as one of the indespensible methods of plant breeding.

Swaminathan (1966) suggested that both kinds of mutagens have been effective in inducing mutations in crop plants. The potential physical mutagens like gamma rays cause severe reshuffling of the genetic material and induce variation of various kind in crop plants (John, 1999). In sexually propagated crops, seeds are treated. Although the mutagenic chemicals are easily available, many geneticists prefer rays. Their main advantage lies in the fact that the seeds are irradiated in a dry condition and that they can be handled for sowing like untreated material. Another disadvantage following application of some of the most potent chemical mutagens is that their negative effects on the vitality and fertility of the M_1 plants are considerably stronger than the corresponding effects of mutagenic rays. This is a very important problem in the practical performance of applied mutagenesis which has not yet been solved.

Widespread occurrence of leaf spot disease in amaranth in Kerala is causing considerable losses. The disease assumes serious proportions in hot humid conditions, leading to severe blighting and drying up of leaves. The causal organism of leaf spot in *Amaranthus tricolor* is *Rhizoctonia solani* (Gokulapalan *et al.*, 1999 and Smitha, 2000). The lack of genes responsible for resistance give a scope for induced alteration in the existing genotype of this particular crop.

Extensive research has been done by various investigators and detailed reviews relating to the induction and recovery of mutations in numerous crops have been presented. Induced mutagenesis on leafy vegetables is very rare. The literature reviewed in this section covers sexually propagated crops in general, emphasizing mainly on vegetable crops including information on amaranth which is very much limited.

The review is presented under the following headings.

1) Estimation of LD_{50} dose
2) Induced mutations in M_1 generation
3) Induced mutations in M_2 generation

2.1 ESTIMATION OF LD_{50} DOSE

Substantial work has been carried out in various crops and the doses of radiation varied from crop to crop. Smith (1972) reported that seeds are the preferred experimental material for mutation breeding. The seeds can be exposed to a wide range of radiation doses and environmental conditions without much physical constraints.

2.1.1 Germination

2.1.1.1 Other crops

The effect of mutagens on germination of crop plants varied markedly. Sen and Datta (1976) reported that when dried seeds of cucumber (*Cucumis sativus* L.) were irradiated with gamma rays or X-rays at 25-55 kR, seed germination percentage was reduced by the highest dosage but was little affected by other doses. Danno *et al.* (1980) while investigating the effects of gamma radiation upto 220 kR on wet and dry seeds of cucumber found that emergence of treated dry seeds was unaffected upto 60 kR, reduced by 20 per cent at 100 kR and inhibited above 220 kR. Reduction in germination of seeds with physical mutagen have also been reported in cowpea (Subramanian, 1980). Narang and Prakash (1983) noticed that as gamma rays increased (5, 10, 15 and 20 kR), germination decreased in *Citrullus lanatus* cultivar Asahi Yamato, *Laganaria siceraria* cv. Pusa Summer. Thirugnanakumar (1986) tried induced mutagenesis in cowpea and found that LD_{50} for germination was 55 kR for gamma rays. Suma Bai and Sunil (1993) found that cultivars of winged bean did not vary significantly with respect to germination percentage. The highest germination percentage was 89.33 per cent at 5 kR, while it was 78.67 per cent on control. The maximum inhibitory effect on germination was at 120 kR which was 44 per cent. Similar reports of reduction in germination in winged bean with increase in dose was observed by Veeresh *et al.* (1995). Animon (1996) in his mutation studies on bhindi concluded that germination percentage was not significantly different at the lowest doses of gamma rays and untreated ones but significantly low at 30 and 40 kR doses in both laboratory and field conditions. The least germination percentage in 40 kR dose was 60 per cent and it was on par with 30 kR dose. Nair (1996) noticed a reduction in germination percentage corresponding to an increase in the dose of gamma rays in lima bean (*Phaseolus lunatus* L.). The highest reduction in germination was noticed in 20 kR treatment. Munishamanna *et al.* (1998) reported that treatment of lima bean seeds with gamma rays resulted in reduction in germination percentage with increase in dose with a definite trend except at 10 kR which showed higher germination.

2.1.1.2 Leafy vegetables

Behera and Patnaik (1979) found that while there was no remarkable variation in the percentage of seed germination in *Amaranthus hypochondriacus* within the range of 10 kR to 40 kR gamma rays, there was a drastic fall with 60 kR. Similar reports of reduction in germination with increased dosages of gamma rays in fenugreek was reported by Sayed (1986). The gamma irradiation significantly reduced the seed germination percentage in *Amaranthus spp.* irrespective of the types at higher dose rates of 75 kR and 100 kR. At lower doses of 10 kR it ranged from 83.64 per cent in A-33 to 98.81 per cent in A-62. At higher doses of 100 kR the germination percentage was 56.42 per cent in A-33.

2.1.2 Survival

2.1.2.1 Other crops

Survival percentage is a better estimate of lethality as it accounts for post germination mortality also. The survival reduction was reported to have a linear relationship with increasing dose rates of gamma rays in cowpea (Subramanian, 1980). The LD_{50} for this crop was found to be around 30 kR according to Mini (1989). Lopez (1990) reported that a dose of 100 Gy led to a slight increase in the value of survival percentage in comparison with the control in Cucumis. Dharan (1993) found that there was reduction in survival of winged bean seedlings with increase in doses of gamma rays. SumaBai and Sunil (1993) noted that the LD_{50} for survival for winged bean was 60 kR. There was a very high significant differences between the dosages, between cultivars and between their combinations. Survival of seedlings decreased progressively with increase in exposure at higher levels. The percentage of survival was higher in 5 kR and 2 kR compared to control. Survival percentage was least at 120 kR. The survival percentage reduced drastically with increase in dosages of gamma rays (Veeresh *et al.*, 1995). Gunasekaran *et al.* (1996) studied the LD_{50} dose for cowpea with gamma rays and found it to be 50 kR. Nair (1996) reported that the highest survival reduction was noticed in 20 kR treatment in limabean. Munishamanna *et al.* (1998) found that the seeds of lima bean did not survive at 30 kR and beyond. The effect of irradiation on seedling survival was more drastic at higher doses. John (1999) found the LD_{50} for Co-4 variety of cowpea to be 40 kR.

2.1.2.2 Leafy vegetables

Behera and Patnaik (1979) reported that with the increase in the doses of mutagens there was a gradual reduction in the percentage of seed germination and percentage of plant survival. A dose requirement of 20-25 kR of gamma rays for survival was reported by Sayed (1986) in fenugreek. Mohideen (1988) observed that there was a linear reduction in survival with an increase in dose from 10-100 kR in all amaranth types. At 100 kR level the seedling survival was nil. The dose which caused 50 percent reduction in survival ranged from 30-40 kR in A-33.

2.2 INDUCED MUTATIONS IN M_1 GENERATION

2.2.1 Effect on morphological characters and yield

2.2.1.1 Other crops

Toxic effects produced by mutagen treatment on biological systems are expressed in the M_1 generation. Ojomo and Omueti (1978) reported that there was no decrease in fertility of plants in M_1 after irradiation with gamma rays at 15, 20 and 25 kR in cowpea. Chowdhury and Singh (1980) found that different genotypes respond differently to the varying doses of irradiation. Nirmala Devi (1982) observed considerable variability in M_1 generation of bhindi for days to flowering, plant height, leaf length and leaf width. Maximum variability was observed for fruit yield per plant for 15 kR. Oomen and Gopimany (1984) tried gamma rays and found that germination, survival, plant height and pollen fertility in cowpea reduced with increasing dose of the mutagen. Abraham (1985) found that gamma rays significantly reduced germination percentage, plant height, number of leaves and fruits, fruit yield and fruit length in M_1 generation of bhindi. Kamannavar (1985) reported that treatment with mutagens in chilli reduced the means of all quantitative characters studied with some exceptions *viz.* days to flowering, plant height, etc. Krishna (1985) observed reduction in crop duration of bhindi in M_1 generation. Thirugnanakumar (1986) reported steady reduction in most characters of cowpea with increase in dose. Jeevanandam *et al.* (1987) indicated a marked reduction effect in plant height at maturity in bhindi. Mini (1989) reported reduction in plant height with increased doses of mutagens in cowpea at the time of harvest. Plant height at flowering was less for all treatments when compared to the control. It decreased with increase in doses of gamma rays. Days to flowering showed an inverse relationship with doses. Dharan (1993) noticed a slight increase in height of winged bean at 100 Gy dose in comparison to control and thereafter an inverse relationship. At lower levels of 100 Gy and 200 Gy an insignificant reduction in number of days to first flowering and a significant increase at 300 Gy and 400 Gy was noticed by her. Fruit yield also decreased significantly with increase in doses of gamma rays. The range of fruit yield per plant was higher in gamma ray treated population, which ranged from 24 per cent at 400 Gy to 105 per cent at 100 Gy in comparison to control. A stimulatory effect on seedling height, number of days to maturity and number of pods per plant at lower doses of 5 and 10 kR and a reduction in the same parameters at higher dosages in lima bean was revealed by Mensah and Eruotor (1993). Ciftci *et al.* (1994) irradiated seeds of french bean with gamma rays and found that increased doses increased the physiological damage and caused significant reduction in plant height, plant weight, pods per plant, seed yield per plant and harvest index. Similar reports by Veeresh *et al.* (1995) in winged bean. Animon (1996) found lower doses of irradiation to be on par with control with respect to the days taken for flowering in bhindi. Plants at 400 Gy dose took highest number of days to flower. He also observed reduction in plant height with increased dosages of gamma rays. There was a significant increase in number of pods per plant at 20 kR of gamma ray in Pusa Komal, which was responsible for slight stimulatory effect in harvest index (Gunasekaran *et al.* 1996). Nair (1996) in her studies on lima bean concluded that lower doses recorded plant height greater than control and higher doses recorded less height. Numbers of branches were also more in lower doses when compared to control. Mohanasundaram *et al.* (1997) found that in M_1 generation the mean values for plant height, number of branches per plant, pod length, pod weight, number of pods per plant, pod yield per plant and harvest index showed significant decrease with increase in doses of gamma rays in cowpea. Delayed flowering was observed in respect of 50 percent flowering in irradiated population. The effect of gamma irradiation on flowering revealed a dose dependent relationship indicating a relationship between dose administered and days taken to flowering. Number of days taken to flowering in lima bean increased with increase in dosages of gamma rays (Munishamanna *et al.*, 1998).

2.2.1.2 Leafy vegetables

Behera and Patnaik (1979) found that there was no remarkable variation in the height of the plants at maturity except those of 60 kR gamma rays treatment which did not survive beyond seedling stage. Sayed (1986) noted that plant height and yield was significantly reduced at higher doses in fenugreek. Mohideen (1988) found stimulatory effect of gamma rays at lower doses on height of plant at maturity. It showed a decreasing trend with increasing dose. Stimulatory effect on yield of greens was noted at lower doses and yield decreased with higher doses. Leaf length decreased significantly with increasing doses. Leaf length differed significantly with varieties and it ranged from 3.37 cm in A-33 to 19.4 cm in A-83. Similar trend was noted for leaf breadth. All treatments had number of branches more than control but showed a decreasing trend with increase in doses.

2.2.2 Morphological variations

In M_1 many workers reported plants showing variation for different morphological characters. Dharan (1993) reported alterations in size and shape of leaves, branching, fasciation, colour in winged bean. Munishamanna *et al.* (1998) found few plants showing variation for different morphological characters *viz.* chimeras, bushy types and seed coat variation.

2.2.2.1 Leafy vegetables

Behera and Patnaik (1979) recorded a wide range of morphological variations in *Amaranthus hypochondriacus* but found that these were not transmissible. All the mutagens affected branching in the shoot at various stages of development. The occurrence of the shoot dichotomy and forked leaf was relatively higher at in gamma ray treatment specially in the 40 kR dose. Mohideen (1988) reported branching mutants high at higher doses. Leaf mutants with broad, small, narrow fasciated variegation were observed in *Amaranthus sp.*

2.2.3 Disease resistance

2.2.3.1 Other crops

Breeding for disease resistance certainly represents the most important way to counteract the pathogens. Induced breeding is now being developed as a complementary tool in breeding for disease resistance. Breeding resistant varieties are in many cases the most economic and the least hazardous measure of managing crop plant diseases. Induced mutation could be useful to develop resistant varieties provided adequate screening techniques to detect the desirable plant characters are at hand (Micke, 1975). About 99 per cent of all mutants are due to recessive mutations. Therefore, the mutant genes are not discernible in the M_1 plants. The selection of mutants can only begin in the M_2 generation (Gottschalk and Wolff, 1983). Literature on induced mutagenesis for disease resistance in amaranth is not available. Hence literature on induced mutagenesis for disease resistance broadly covers all crops, which are sexually propagated. The first report on the induction of mutations for disease resistance is by Friesleben and Lein (1942). Working in Germany they isolated a mutant in Haisa Barley simultaneously resistant to three races of Powdery Mildew, as a result of treatment with X-rays. Malik (1991) developed two mutants resistant to mung bean yellow mosaic Gemini virus and Cercospora leaf spot (*Mycosphaerella cruenta*) following gamma irradiation of hybrid seeds. Venkatachalam and Jayabalan (1997) subjected two week old calluses with multiple shoot buds to gamma irradiation (50, 100, 150, 200 and 250 Gy) and transferred to a medium containing *Phaeoisariopsis personata* (*Mycosphaerella berkeleyi*) culture filtrates. Of the 83 progenies screened, 73 were considered susceptible (PDI = 65-85%) and 10 progenies were considered resistant (PDI = 30-50%). On mutagenesis with EMS @ 0.5 per cent three early maturing foliar disease (leaf spot) resistant mutants in groundnut were isolated (Motagi *et al.*, 2000).

2.2.4 Effect on biochemical attributes

2.2.4.1 Other crops

Khalil and Moursy (1976) observed increase in the total nitrogen (N) content of seedlings and phosphorus content of plants at the preflowering stage following combined treatments of squash (*Cucurbita pepo* L.) seeds with 0.5, 2.5 or 5.0 kR gamma irradiation and soaking in solution containing 0, 20, 100 or 200 mg/lit. IAA. Mutations affecting morphology and chemical composition were also obtained by Sanoev and Zorina (1977) in cucumber following irradiation with 10-15 kR gamma rays. Rajasekharan and Shanmugavelu (1984) isolated a mutant in *Momordica* *charantia* L. following gamma irradiation. It was early flowering and fruits were rich in ascorbic acid, protein, phosphorus, potassium and calcium. Tripathi *et al.* (1991) reported the inductive effect of ultraviolet exposure in increasing the protein, phosphorus and total sulphur content in seeds of green gram. Mensah and Eruotor (1993) in their studies on lima bean found that there was little or no effect of irradiation on protein content at lower dosages of 5 and 10 kR and a progressive decrease with increasing dosages.

2.3 INDUCED MUTATIONS IN M_2 GENERATION

2.3.1 Micromutations

The expression micromutation is used to mean mutations in polygenes governing quantitative characters, leading to small changes in phenotypes. Majority of the economically important characters in crop plants are polygenic in nature. Following the successful experiences of Gregory (1955) in the usefulness of mutation tool for groundnut improvement, breeders in different crop plants resorted to the micromutation technique to improve quantitatively inherited characters like yield and its components.

2.3.1.1 Other crops

Swaminathan (1966) reported that micromutation could be identified only through biometrical procedures. There was a progressive decline in mean values for most of the characters in the M_2 generation in bhindi depending on dose (Abraham, 1985). A variety dependent significant variation in plant height was noticed in chilli when exposed to gamma rays (LekhaRani, 1985). But there was no significant variation among varieties, between mutagens, between levels of mutagens, treatments compared to control and variety into dose effect interactions for number of branches and yield per plant. Quintero *et al.* (1990) reported that the diversity of crop patterns among the cowpea mutants with superior yield over the parents in M_2 generation was due to variations of combinations of increased number of fruiting branches, rate of leaf production and larger duration of photosynthetic area. Raju (1994) observed significant differences in mean yield among the varieties of cucumber and also among the different doses of gamma rays. Interaction between varieties and gamma ray doses were also found to be statistically significant. Gunasekaran *et al.* (1996) found that the plant height on 30th day, pods per cluster and 100 seed weight remained unaffected in cowpea. Changes towards positive and negative direction of mean were observed for days to 50 percent flowering and yield per plant. Mean values for plant height at maturity in lima bean was found to have been shifted towards negative direction (Nair, 1996). John (1997) noticed significant difference in plant height and fruit weight per plant among the treatments in bhindi. Maximum fruit weight of plant was observed in the treatment 20 kR and the least in the unirradiated treatment. Crop duration was the shortest for control and maximum for the treatment 40 kR. In M_2 generation, plant height, number of branches per plant, length of pod, weight of pod, number of pods per plant, pod yield per plant and harvest index showed positive change at lower doses and negative change at higher doses in cowpea (Mohanasundaram *et al.*, 1997). He observed a significant reduction in germination and survival percent in cowpea with increase in doses of gamma rays. Similar results have been reported by Senthilkumar and Natrajan (1997) in bhindi and John (1999) in cowpea.

2.3.1.2 Leafy vegetables

Mohideen (1988) found that there was significant difference for plant height among the treatments in amaranth. There was a negative shift in most of the quantitative characters studied with increase in doses of gamma rays.

2.3.2 Viable mutations

A wide spectrum of viable and macromutations have been reported for plant type, flower, leaf, seed size and yield in various crops.

2.3.2.1 Other crops

Subramanian (1980) in *Vigna sinensis* reported a large number of plant and leaf abnormalities viz. tall, dwarf, leaf shape mutants, pyramid, bushy and sterile mutants. Calcagno *et al.* (1984) isolated many leafy, erect and late types of potential agronomic interest in Fenugreek with gamma irradiation with 15 and 20 kR.

Filippette and Marzano (1984) obtained a determinate mutant with ten inflorescence below the terminal one on the main stem and a dwarf mutant both of which were considered of potential value in *Vicia faba.* Vasudevan *et al.* (1984) obtained two non training erect and two semi-erect mutants of fodder cowpea in M_2 generation. Though erect mutants showed reduction in plant height, the green yield per plant was higher per plant due to more branches. He also obtained one early mutant through 20 kR acute gamma irradiation and this mutant flowered and matured 35 and 25 days earlier than control. Haq *et al.* (1989) isolated a very early flowering mutant in cowpea at 20 kR gamma rays and found that the trait was stable in M_3 to M_6 generation. In the study conducted by Nair (1996) in lima bean, viable mutants altering plant habit, leaf morphology, duration of flowering and pod size were observed. Mutation frequency was high at 16 to 20 kR in lima bean. Viable mutants like dwarf plants, early maturing and high yielding plants were obtained. Singh and Singh (1998) observed more branching, excessive dwarfness, broad leaf, long fruits, early and late flowering as well as high yielding types of mutants of okra in certain doses of gamma rays and EMS.

2.3.2.2 Leafy vegetables

Behera and Patnaik (1979) obtained fasciated and curled leaf mutants with chemical and physical (gamma rays) mutagens. The histological analysis of the mutants suggested that the fasciation resulted due to flattening and enlargement of growing points and leaf curling due to irregular development of lamellar vascular bundles and enlargement of adjacent tissues. Hadke (1981) isolated a mutant with 2-5 per cent EMS in spinach with a long vegetative phase. Calcagno *et al.* (1984) isolated many leafy, erect and late types of potential agronomic interest in fenugreek with gamma irradiation with 15 and 20 kR. Sayed (1986) reported that frequency of viable mutations increased with an increase in the dose rates in fenugreek. Mohideen (1988) obtained early maturity mutants in amaranth at higher doses. Branching mutants were obtained at higher doses. Leaf mutants broad, small, narrow and fasciated were obtained.

2.3.3 Effect on disease resistance

2.3.3.1 Other crops

Induced mutations for specific characters such as disease resistance and quality aspects in crop plants occur generally at low frequency. Doligkh and Korganova (1974) obtained cucumber mutants resistant to *Cladosporium cucumerinum* after treatment with 0.02 per cent EI and 0.5 and 1 kR of fast neutrons. Thandapani *et al.* (1978) released a high yielding mutant bhindi, MDU-2 produced by treating seeds of Pusa Sawani with 0.04 per cent of diethyl sulfoxide. The mutant showed a higher level of resistance to yellow vein mosaic disease than Pusa Sawani under field conditions during winter. A mutant similar to *Abelmoschus tetraphyllus* was isolated with resistance to yellow vein mosaic disease from the M_2 generation of irradiated *A. esculentus* varieties (Abraham, 1985). Shaikh (1988) reported that gamma irradiation of mungbean yielded mutants with 15-17 per cent higher yields and resistance to mungbean yellow mosaic gemini virus and Cercospora leaf spot disease. Sharma *et al.* (1989) isolated two leaf rust resistant mutants in wheat. Dryseeds of mung bean were exposed to gamma rays by Yadav and Singh (1991) and a number of mungbean yellow mosaic resistant lines were identified in later generations from M_2. In a mutation breeding programme initiated due to non availability of sources of resistance in Sri Lanka to blight in sesame, gamma ray treatments of 450 and 600 Gy produced more lines tolerant to the disease than the other doses used (Pathirana, 1992). Sheela (1994) reported that a radiation dose of 50 kR on the hybrid seeds of interspecific crosses between *A. caillei* and *A. tetraphyllus* with *A. esculentus* was optimum for inducing breakage of linkage and expression of variability. Kalia *et al.* (2000) isolated mutant plants having better rust resistance in wheat in M_2 and M_3 generations with effective mutagenesis. Singh and Singh (2000) obtained highly resistant plants in okra in both M_2 and M_3 generations. Incidence of yellow vein mosaic virus showed dose dependent relationship and increase in doses of mutagens decreased the disease infection.

2.3.4 Effect on biochemical attributes

2.3.4.1 Other crops

Mirchev and Nedelcheva (1974) reported increased crude protein and carotene content in lucerne at a dose of 1300 R of gamma rays. In *Phaseolus vulgaris,* phytic acid is able to chelate minerals, thus reducing the availability of them in animal nutrition. Fifty varieties from induced mutations with different content of phytic acid were identified by Lolas and Markakis (1975). Mutants in *Lathyrus sativus* are known in which BOAA (neurotoxin) is reduced (Gottschalk and Wolff, 1983). Ignacimuthu and Babu (1989) suggested that the range of induced variation in the whole seed protein of urdbean and mungbean was narrow within the species but within each mutant line, the range was very broad. Some of these high protein mutants have high levels of lysine and limiting S-amino acids. Pillai and Abraham (1996) obtained a sweet pepper mutant on treating with 0.5 per cent EMS that had significantly increased amounts of vitamin C. Mohanasundaram *et al.* (1997) noted a stimulatory effect on lower dose of 20 kR in case of protein content. Kalia *et al.* (2000) isolated mutants with high protein content in wheat.

A good source of resistance to leaf spot disease has not been identified so far among the cultivated amaranth varieties. By use of radiation technique, it may be possible to increase the genetic variability so that a mutant resistant to leaf spot may be identified. The present study is an attempt to see the effect of gamma rays on disease resistance in amaranth and induce desirable mutants in later generations.

Materials and Methods

3. MATERIALS AND METHODS

The present study was undertaken in the Vegetable Research Field of the Department of Olericulture, College of Horticulture, KAU, Vellanikkara, Thrissur during October 2001 to October 2002. The location is situated at an attitude of 22.25 m, 10° 32'N latitude and 76°16'E longitude with a typical warm humid tropical climate. The soil type is deep, well drained sandy loam with pH 5.1.

3.1 MATERIALS

The details of the materials used are presented below.

3.1.1 Biological material

Two genotypes viz. Arun and A-204 belonging to two species (Plate 1) viz., *Amaranthus tricolor* and *A. hypochondriacus* respectively were selected for the study. Main morphological characters of the two Amaranth species are given in Table 1.

3.1.2 Mutagens

The physical mutagen employed for the study was gamma rays. The gamma irradiation facilities available at the Radio Tracer Laboratory, College of Horticulture, Kerala Agricultural University, Vellanikkara were utilized, CO_{60} gamma cell unit was employed for irradiation.

3.2 METHODS

The details of the methods followed in this study are presented under the following heads:

1) Standardization of the optimum dose of the physical mutagen for different Amaranth species.
2) Effect of gamma rays on morphological, biometrical characters and on leaf spot disease in M_1 generation.
3) Effect of gamma rays in M_2 generation and selection of desirable mutants from the population.

Table 1. Morphological description of the two amaranth species

Sl. No.	Characters	*A. tricolor* (Arun)	*A. hypochondriachus* (A-204)
1	Terminal inflorescence characters		
1.1	Laterals	Absent	Present
1.2	Shape	Spike	Panicle
1.3	Latitude	Erect	Erect
1.4	Colour	Red	Green
2	Axillary inflorescence	Present	Present
3	Leaf characters		
3.1	Pubescence	Nil	Nil
3.2	Pigmentation	Red	Green
3.3	Shape	Elliptical	Lanceolate
3.4	Prominence of vein	Smooth	Smooth
3.5	Margin	Undulate	Entire
4	Stem characters		
4.1	Petiole pigmentation	Red	Green
4.2	Stem pigmentation	Red	Green
4.3	Pubescence	Nil	Nil
4.4	Growth habit	Erect	Erect

A. tricolor (Arun)

A. hypochondriacus (A - 204)

Plate 1. Amaranth species selected for the study

3.2.1 Standardisation of the optimum dose of the physical mutagen for different Amaranth species

3.2.1.1 Estimation of LD_{50}

One hundred selfed and well filled seeds of uniform size of each species were irradiated with different doses of gamma rays (100, 200, 300, 400, 500, 750 and 1000 Gy) and sown in pots replicated two times and the following observations were recorded.

a) Germination (%)

The emergence of seedlings at five days interval for two weeks were recorded and expressed as percentage.

b) Survival (%)

The number of seedlings with atleast one pair of true leaves on 30^{th} day, were counted and expressed as percentage.

3.2.1.2 Standardizing the optimum dose of the mutagen

Based on the result obtained from the estimation of LD_{50}, eight doses of gamma rays at regular intervals (50, 100, 150, 200, 250, 300, 350 and 400 Gy) with LD_{50} as the highest dose were fixed for further field trials.

3.2.2 Effect of gamma rays in M_1 generation

Seeds of each genotype were exposed to eight doses of gamma rays and were sown in field along with the control in a Randomised Block Design with three replication. Three weeks old seedlings were transplanted in the main field. Planting was done at a spacing of 30 x 20 cm^2. The crop was raised during October 2001 to as per the Package of Practices Recommendations for crops (KAU, 1996). There were 30 plants per treatment per replication. The growth observations were recorded on ten randomly selected plants from each treatment of each replication of both the Amaranth species. The following are the growth observations recorded in M_1 generation

3.2.2.1 Germination (%)

3.2.2.2 Survival (%)

3.2.2.3 Plant height at first harvest (cm)

The height was recorded from ground level to the tip of the stem on 30th day after transplanting.

3.2.2.4 Plant height at flowering (cm)

Height was measured from ground level to the tip of the terminal spike.

3.2.2.5 Branches per plant

The number of branches per plant was counted at the time of flowering.

3.2.2.6 Leaf length and leaf width (cm)

The fifth leaf from the terminal bud was selected and length was measured from base of leaf to the tip along the central vein. The width of the leaf was measured at the region of maximum width.

3.2.2.7 Days to 50 per cent bolting

From the plants kept unharvested, days to 50 per cent bolting were recorded.

3.2.2.8 Leaf stem ratio

Both leaf yield and stem yield were recorded separately to calculate the leaf stem ratio.

3.2.2.9 Vegetable yield (g/plant)

Ten plants were randomly selected for recording vegetable yield at each harvest. First harvesting was done on the 30th day of transplanting and then at an interval of fifteen days. The weight of greens from different harvests constitute vegetable yield per plant.

3.2.2.10 Crop duration (days)

Duration of crop from the date of sowing till harvest of seeds was recorded.

3.2.2.11 Disease incidence (%)

Observations on disease incidence and intensity/severity in the main field were recorded at 15th, 30th and 45th days after transplanting. For scoring the disease five plants were tagged per treatment per replication. Five leaves starting from the top 3rd to 7th leaf towards the base were recorded each time.

The percentage disease incidence was calculated by using the formula,

$$\text{Disease Incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

3.2.2.12 Disease severity (%)

Disease severity was rated as per the scoring system given below.

Disease score	Percentage of leaf area infected
1	Nil
2	1-10
3	10.1-25
4	25.1-50
5	> 50

Based on the percentage of leaf area infected, disease severity/intensity was calculated using the following formula (Wheeler, 1969).

$$\text{Percentage disease severity/ intensity (PDI)} = \frac{\text{Sum of all numerical rating} \times 100}{\text{Total No. of leaves taken for observations} \times \text{Maximum disease grade}}$$

Based on percentage disease severity, the treatments were grouped into five categories as suggested by Rajkumar *et al.* (1995) with slight modifications, which are as follows:

Disease severity (%)	Disease score	Category
0	1	Immune
0.1-10	2	Highly resistant
10.1-25.0	3	Moderately resistant
25.1-50.0	4	Moderately susceptible
>50	5	Highly susceptible

3.2.2.13 Morphological variations, if any

Observations on any morphological variations from the normal plant were noted.

3.2.2.14 Beta carotene (I.U)

Beta carotene was estimated by A.O.A.C. (1970) using saturated n-butanol.

3.2.2.15 Vitamin C (mg/100 g)

Vitamin C of fresh samples was estimated by method of A.O.A.C. (1955) using 2,6 dichlorophenol indophenol dye.

3.2.2.16 Oxalate (%)

Oxalate content of the sample was estimated calorimetrically using iron ferron reagent as suggested by Marderosian *et al.* (1980).

3.2.2.17 Nitrate (%)

Nitrate content was analysed calorimetrically using diphenol sulphonic acid as suggested by Bhargava and Raghupathi (1993).

3.2.3 Study of M_2 generation

Selfed seeds were collected from five plants per treatment and sown in the field along with the control to study variability in M_2 generation. There were 50 plants per treatment per replication. The crop was raised during July 2002 to October 2002.

3.2.3.1 Micromutations

Detailed observations on the following quantitative traits were made and data collected.

a) Germination (%)
b) Survival (%)
c) Plant height at first harvest (cm)
d) Plant height at flowering (cm)
e) Branches per plant
f) Leaf length (cm)
g) Leaf width (cm)
l) Days to 50 per cent bolting
i) Vegetable yield (g/plant)
j) Leaf stem ratio
k) Crop duration (days)
l) Disease incidence (%)
m) Disease severity (%)
n) Beta carotene (I.U)
p) Vitamin C (mg/100 g)
q) Oxalate
r) Nitrate

For vegetable yield in M_2 generation, 30 plants were taken for recording the observation and the yield per plant was worked out.

3.2.3.2 Viable mutations

Gamma ray treated and control plants were subjected to periodical observations and the visual variants were scored.

3.2.4 Statistical analysis

The data recorded from the pot experiment was analysed in a completely randomized design (CRD) using ANOVA. For field experiments, ANOVA was done in factorial RBD using MSTAT C software package with two species and nine treatments (control and eight doses of gamma rays) in three replications to find out the significant differences among the treatments in respect of the various characters.

Results

4. RESULTS

The results of the study entitled 'Induction of leaf spot resistance and improvement in quality of amaranth through mutation' are presented under the following headings:

1. Standardisation of the optimum dose of the physical mutagen for different Amaranth species
2. Effect of gamma rays in M_1 generation
3. Effect of gamma rays in M_2 generation and selection of desirable mutants from the population

4.1 STANDARDISATION OF THE OPTIMUM DOSE OF THE PHYSICAL MUTAGEN FOR DIFFERENT AMARANTH SPECIES

4.1.1 Estimation of LD_{50}

The LD_{50} was found out based on germination percentage and survival percentage. The results of germination and survival percentage are presented below.

a) Germination percentage

A progressive decrease in the germination percentage was observed with increasing doses of gamma rays. The germination percentage under various treatments in different species of amaranth is presented in Table 2 and graphically represented in Fig.1. Significant variation in germination was observed at various levels of gamma rays. Maximum germination was observed in control (83.5%) and least in seeds treated with 1000 Gy of gamma rays (18%) in the species *Amaranthus tricolor* (Arun). In *A. hypochondriacus*, lower doses (100 Gy and 200 Gy) were on par with control with a germination percentage of around 80 per cent. In each treatment, germination percentage was higher in the species *A. hypochondriacus* than in *A. tricolor*. The relative percent over control for seed germination was 50 per cent for 500 Gy in *A. tricolor* and 750 Gy in the case of *A. hypochondriacus*.

Table 2. Effect of gamma rays on seed germination for estimation of LD_{50}

Dose (Gy)	*A. tricolor* (Arun)		*A. hypochondriacus*	
	Germination (%)	Relative percent over control	Germination (%)	Relative percent over control
0	83.50	100.00	81.00	100.00
100	72.00	86.23	79.00	97.53
200	60.00	71.86	80.00	98.76
300	64.00	76.65	71.00	87.65
400	61.00	73.05	62.00	76.54
500	42.50	50.89	49.50	61.11
750	33.00	39.52	40.50	50.00
1000	18.00	21.56	19.50	24.07
Mean	54.24		60.31	
SE	1.68		2.39	
CD (0.01)	5.47		7.79	

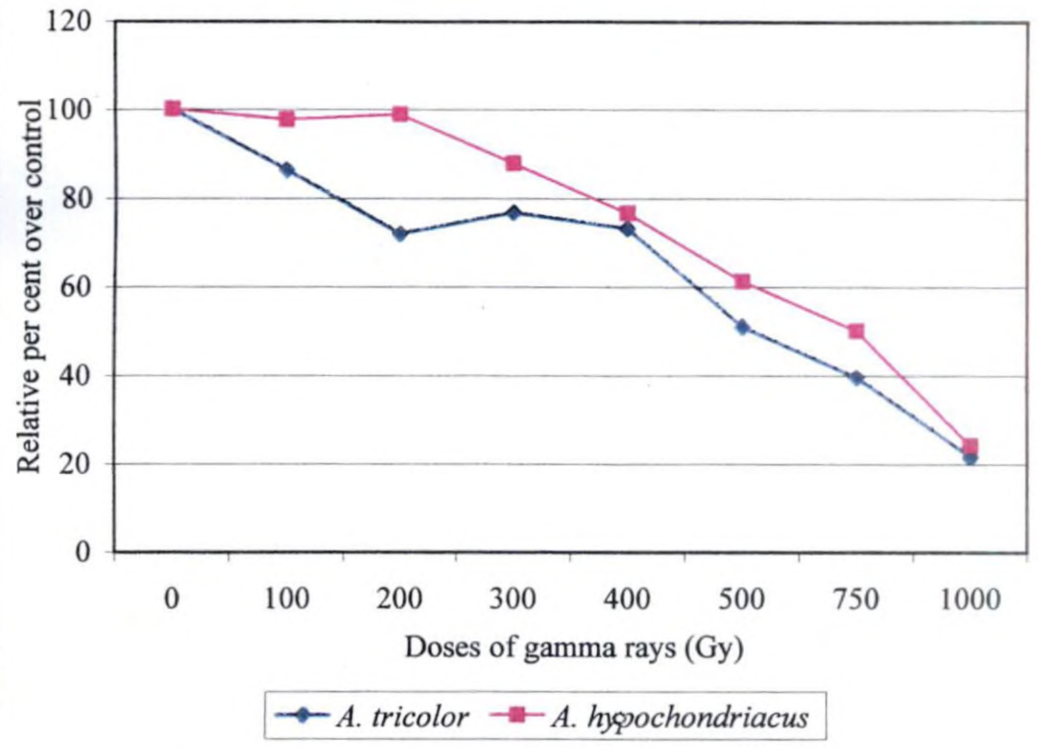


Fig. 1. Effect of gamma rays on seed germination for estimation of LD_{50}

b) Survival percentage

The data on the survival of plants in the pots on the thirtieth day after sowing is given in Table 3 and graphically represented in Fig.2. There was significant difference between the treatments for survival percentage. In general, the percentage of survival was observed to decrease with increase in the doses of gamma rays. In *A. tricolor* (Arun), control showed highest survival percentage (80%) while in *A. hypochondriacus*, a survival percentage of around 77 per cent was observed for 100 Gy, which was on par with control. Survival percentage was below 50 in the three treatments starting from 500 Gy and above in both the species studied. Not a single plant survived in the treatments 750 Gy and 1000 Gy in *A. hypochondriacus*. In *A. tricolor* (Arun) a minimum of 10 per cent survival was observed in 750 Gy and no plants survived in 1000 Gy.

Considering the survival percent, 400 Gy was fixed as the LD_{50} for amaranth in the two species studied.

4.2 EFFECT OF GAMMA RAYS IN M_1 GENERATION

4.2.1 Effect of gamma rays on seed germination and survival under field conditions

a) Germination percentage

The effects of gamma rays on the germination percentage under field conditions are presented in Table 4 and graphically represented in Fig.3. There was significant difference between the treatments irrespective of the species. The maximum germination percentage was observed for control (80.33%), which was on par with the treatment 50 Gy and minimum for 400 Gy (53.17%). In general, a decrease in germination was noticed with increase in doses of gamma rays.

b) Survival percentage

The results on the survival percentage of plants under field conditions are given in Table 5 and graphically represented in Fig.4. Various doses of gamma rays

Table 3. Effect of gamma rays on survival percentage for estimation of LD_{50}

Dose (Gy)	*A. tricolor* (Arun)		*A. hypochondriacus*	
	Survival (%)	Relative percent over control	Survival (%)	Relative percent over control
0	80.00	100.00	78.00	100.00
100	70.00	87.50	76.50	98.08
200	56.00	70.00	74.00	94.87
300	59.00	73.75	64.50	82.69
400	58.00	72.50	56.00	71.79
500	35.00	43.75	38.00	48.72
750	10.00	12.50	0.00	0.00
1000	0.00	0.00	0.00	0.00
Mean	46.00		48.37	
SE	1.73		1.09	
CD (0.01)	5.64		3.55	

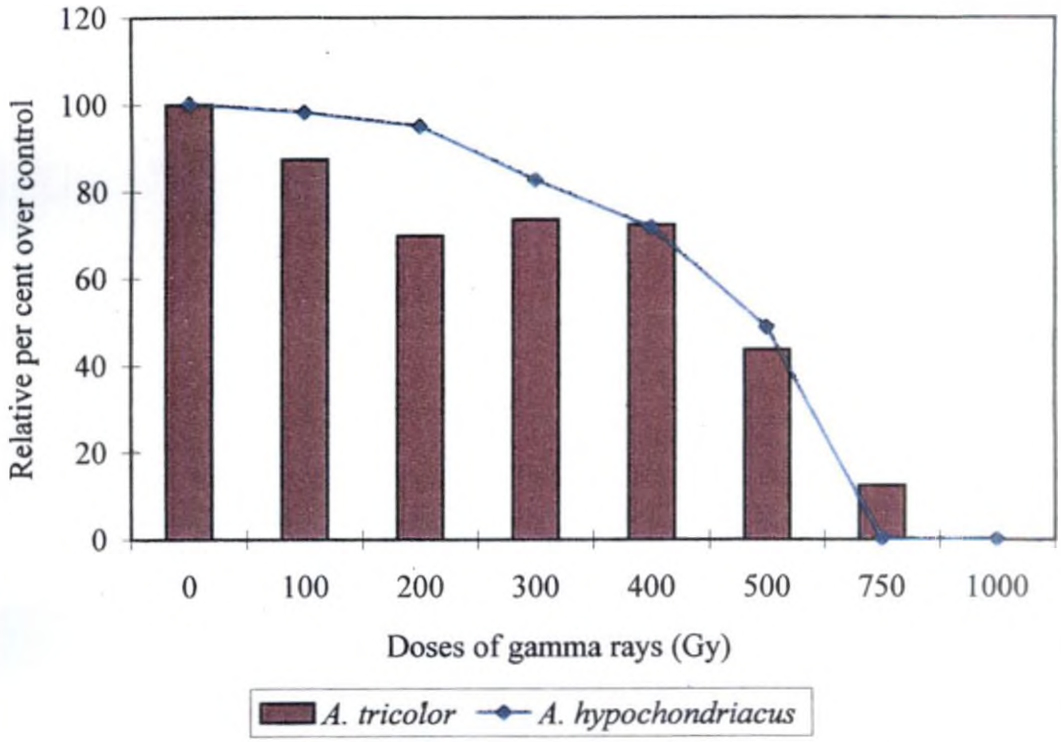


Fig. 2. Effect of gamma rays on survival percentage for estimation of LD_{50}

Table 4. Effect of gamma rays on seed germination in M_1 generation

Dose (Gy)	*A. tricolor* (Arun)		*A. hypochondriacus*		Mean
	Germination (%)	Relative percent over control	Germination (%)	Relative percent over control	
0	78.67	100.00	82.00	100.00	80.33
50	79.33	100.84	73.67	89.84	76.50
100	74.67	94.91	70.67	86.18	72.67
150	72.33	91.94	69.67	84.96	71.00
200	61.33	77.96	65.00	79.27	63.16
250	63.67	80.93	61.00	74.39	62.33
300	63.00	80.08	56.00	68.29	59.50
350	57.00	72.45	53.67	65.45	55.33
400	52.67	66.95	53.67	65.45	53.17
Mean	66.96		65.04		65.99
Between treatments	CD (0.01)				6.99
Interaction					NS

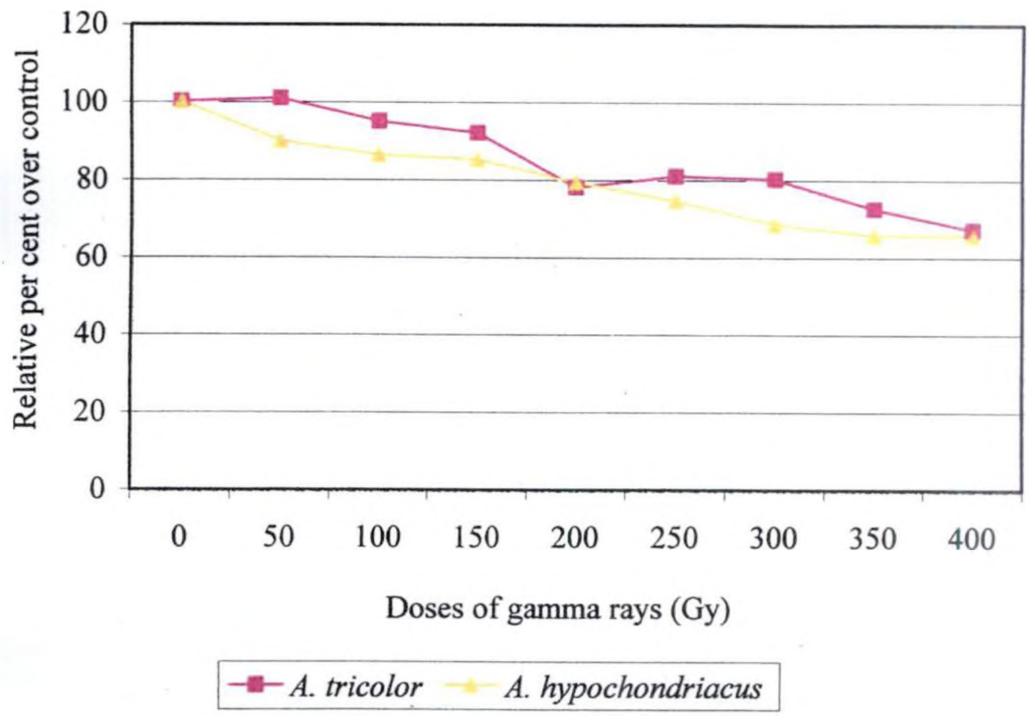


Fig. 3. Effect of gamma rays on germination percentage in M_1 generation

Table 5. Effect of gamma rays on survival percentage in M_1 generation

Dose (Gy)	*A. tricolor* (Arun)		*A. hypochondriacus*		Mean
	Survival (%)	Relative percent over control	Survival (%)	Relative percent over control	
0	75.33	100.00	75.67	100.00	75.50
50	73.33	97.34	66.67	88.11	70.00
100	64.33	85.39	66.00	87.22	65.16
150	64.33	85.39	62.00	81.93	63.16
200	56.00	74.34	58.00	76.65	57.00
250	56.67	75.23	57.33	75.76	57.00
300	58.67	77.88	51.33	67.83	55.00
350	51.33	68.14	50.33	66.51	50.83
400	50.00	66.37	48.67	64.32	49.33
Mean	61.11		59.55		60.33
Between treatments	CD (0.01)				6.13
Interaction					NS

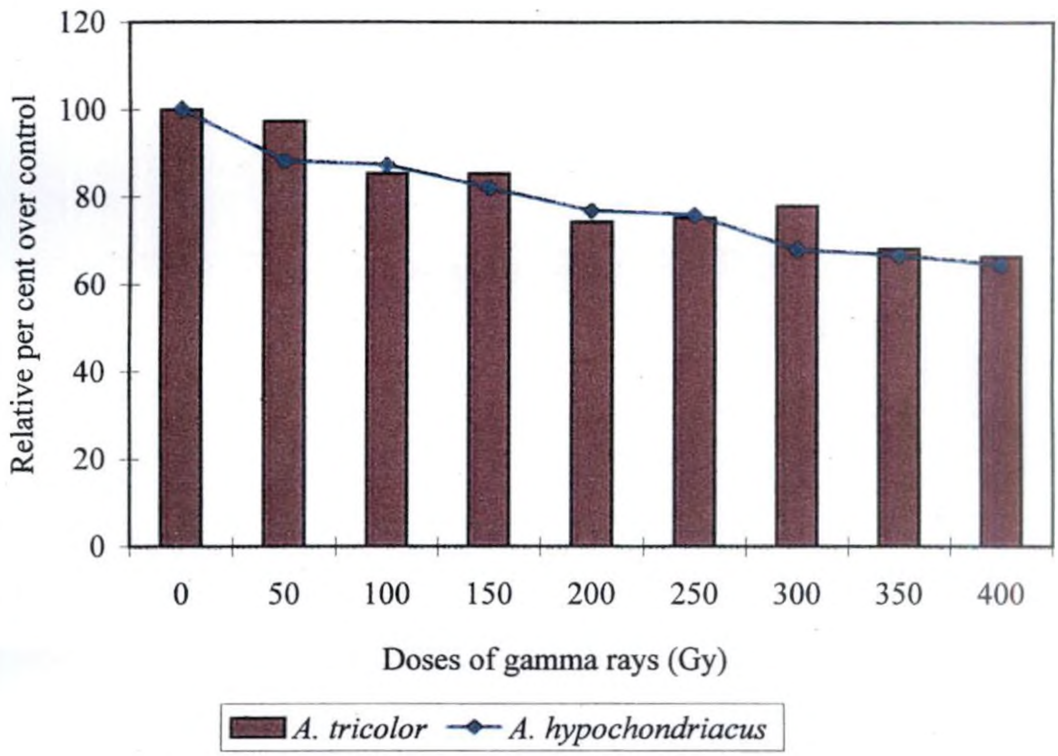


Fig. 4. Effect of gamma rays on survival percentage in M_1 generation

showed significant variation in survival irrespective of the species. The mean survival percentage was the highest in control (75.5%), which was on par with 50 Gy and lowest in 400 Gy dose (49.33%). The effect of 200 Gy, 250 Gy and 300 Gy were on par for survival percentage.

4.2.2 Effect of gamma rays on morphological characters

4.2.2.1 Plant height at first harvest

Various doses of gamma rays caused significant variation in plant height at harvest. The mean plant height at first harvest showed maximum height for 50 Gy dose (50.07 cm), which was on par with control and 150 Gy dose and thereafter an inverse relationship was noticed (Table 6). The treatments differed among themselves within the species. Generally *A. hypochondriacus* was taller than *A. tricolor*. In *A. hypochondriacus*, maximum plant height was observed at 50 Gy (63.6 cm) and for *A. tricolor*, untreated plants showed maximum height (39.2 cm).

4.2.2.2 Plant height at flowering

The analysis of variance for the two species indicated significant difference for plant height at flowering (Table 6). There was significant difference between the treatments and within the species. Mean plant height for the two species showed that the doses 50 Gy and 150 Gy were on par with control and height decreased significantly with increase in dose of gamma rays. Plant height at flowering in *A. hypochondriacus* ranged from 68.00 cm in 350 Gy to 84 cm in 150 Gy. In *A. tricolor*, the plant height varied from 41.3 cm in 400 Gy to 56.8 cm in control.

In *A. tricolor*, plant height for control was on par with 50 Gy dose and then decreased significantly from 100 Gy onwards. Maximum plant height in *A. hypochondriacus* was observed at 150 Gy (84.07 cm) which was on par with control which was followed by the treatments 50 Gy, 100 Gy, 200 Gy and 250 Gy

Table 6. Effect of gamma rays on plant height in M_1 generation

Dose (Gy)	Plant height at harvest (cm)			Plant height at flowering (cm)		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	39.20	52.47	45.83	56.80	79.77	68.28
50	36.53	63.60	50.07	53.13	76.80	64.97
100	32.47	56.67	44.57	45.60	76.27	60.93
150	32.47	58.13	45.20	46.03	84.07	65.06
200	33.47	54.07	43.77	43.43	71.73	57.58
250	30.60	52.40	41.50	43.32	71.73	57.53
300	30.67	49.87	40.27	43.50	68.53	56.02
350	30.00	49.73	39.87	42.43	68.00	55.22
400	28.13	48.53	38.33	41.33	69.40	55.37
Mean	37.60	53.94	43.27	46.17	74.03	60.10

Between treatments	CD (0.01)	5.15	CD (0.01)	5.82
Interaction	CD (0.05)	5.38	CD (0.05)	6.09

4.2.2.3 Branches per plant

Number of branches per plant was significantly different among the treatments irrespective of the species. It showed a decreasing trend with increase in dosages of gamma rays. The mean number of branches per plant was lower for untreated plants and higher for lower doses of 50, 100, 150 and 200 Gy which were on par. Number of branches ranged from 6.35 in 350 Gy to 9.43 in the treatment 200 Gy. The mean number of branches was more in *A. tricolor* when compared to *A. hypochondiacus* (Table 8).

4.2.2.4 Leaf length

The treatments differed significantly for leaf length (Table 7). Mean length of leaf was observed to be higher in lower doses of 50 Gy to 200 Gy. There was no significant difference between the treatments within the species. The maximum mean leaf length was 14.19 cm in 100 Gy, which was on par with 50 Gy, 150 Gy and 200 Gy dose while the minimum leaf length of 10.07 cm was observed for 400 Gy. The treatments 250 Gy (12.57 cm) and 300 Gy (11.98 cm) were on par with control (12.98).

4.2.2.5 Leaf width

The treatments differed significantly for leaf width among themselves. The maximum mean leaf width was observed as 7.16 cm for 50 Gy which was on par with the treatments 100 Gy, 150 Gy and 200 Gy while the minimum mean leaf width of 5.56 cm was observed at 400 Gy dose. Width of leaf was higher in *A. tricolor* (8.28 cm) when compared to *A. hypochondriacus* (4.77 cm) as can be observed from Table 7.

4.2.2.6 Leaf stem ratio

Lower doses of irradiation (50, 100 and 150 Gy) were found to be on par with control for mean leaf stem ratio of both the Amaranth species (Table 7). Least leaf stem ratio was observed for 300 Gy dose (0.88), which was on par with the

Table 7. Effect of gamma rays on branches per plant, leaf length and leaf width in M_1 generation

Doses (Gy)	Branches per plant			Leaf length (cm)			Leaf width (cm)		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	11.20	5.13	8.17	12.47	13.50	12.98	8.26	4.79	6.52
50	11.20	5.93	8.57	12.80	14.40	13.60	8.97	5.35	7.16
100	11.60	5.47	8.53	14.09	14.31	14.19	9.11	5.17	7.14
150	12.53	5.93	9.23	12.62	14.87	13.74	8.67	5.10	6.89
200	11.87	7.00	9.43	12.97	15.23	14.10	8.92	5.15	7.03
250	10.47	4.67	7.57	11.77	13.37	12.57	7.83	4.55	6.19
300	10.53	4.20	7.37	11.53	12.43	11.98	7.73	4.49	6.11
350	8.40	4.30	6.35	10.35	12.51	11.43	7.93	4.3	6.12
400	9.47	3.87	6.67	9.20	10.93	10.07	7.07	4.05	5.56
Mean	10.81	5.17	7.99	11.97	13.50	12.74	8.28	4.77	6.52
Between treatments CD (0.01)			1.23	CD (0.01)		1.19			0.58
Interaction			NS			NS			NS

remaining doses. There was no significant difference between the treatments within the species.

4.2.2.7 Days to 50 per cent bolting

Significant difference for number of days to 50 per cent bolting was observed among the treatments and within the species (Table 8). In general, a progressive increase in days to bolting with increase in doses was observed in both species. All doses except 50 Gy, 100 Gy and 200 Gy showed late bolting. Maximum days to bolting of around 55 days was observed for control and 150 Gy dose which were on par. Minimum days to bolting was seen in 50 Gy, 100 Gy and 200 Gy with a value of around 52 days. *A. hypochondriacus* took more days to bolting compared to *A. tricolor* (Arun). In *A. hypochondriacus*, days to bolting ranged from a minimum of around 57 days in 50, 100 and 200 Gy dose and maximum of 61 days in 400 Gy dose which was on par with the untreated plants, and the remaining doses. Control and 150 Gy dose showed highest days to bolting of around 51 days compared to the rest of the doses which showed 47 to 48 days to bolting in *A. tricolor* (Arun).

4.2.2.8 Vegetable yield (g/plant)

The results presented in Table 9 revealed that there was significant difference between the treatments for vegetable yield per plant. Significant differences for treatments within the species were also noticed. In general, there was a progressive decline in yield with increase in doses. The mean vegetable yield for both the species showed that lower doses had higher yield per plant than control (205 g/plant) and remaining doses. However, in *A. tricolor* (Arun), it was found that yield was significantly higher than control in doses of 50 Gy, 150 Gy and 200 Gy. Yield was maximum at 50 Gy (305 g/plant) and minimum at 400 Gy dose (228 g/plant). In *A. hypochondriacus*, yield was lower than *A. tricolor* (Arun). Vegetable yield was maximum at 150 Gy with a value of 197 g/plant which was on par with control and all doses upto 300 Gy and it was minimum for 350 and 400 Gy with a yield of around 151 g/plant in the case of *A. hypochandriacus*.

Table 8. Effect of gamma rays on leaf stem ratio and days to 50 per cent bolting in M_1 generation

Dose (Gy)	Leaf stem ratio			Days to 50 per cent bolting		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	1.16	0.93	1.04	50.67	59.67	55.17
50	1.35	0.94	1.14	48.33	57.00	52.67
100	1.28	1.09	1.18	48.33	57.33	52.83
150	1.11	0.84	0.97	51.33	59.33	55.33
200	0.88	1.00	0.94	47.67	56.67	52.17
250	0.94	0.88	0.91	48.33	59.33	53.83
300	0.91	0.85	0.88	47.33	59.33	53.33
350	0.96	0.89	0.92	47.33	61.33	54.33
400	0.98	0.88	0.93	48.00	61.33	54.67
Mean	1.06	0.92	0.99	48.59	59.03	53.81
Between treatments CD (0.01)			0.22	CD (0.01)		2.37
Interaction			NS	CD (0.05)		3.35

Table 9. Effect of gamma rays on vegetable yield and crop duration in M_1 generation

Dose (Gy)	Vegetable yield (g/plant)			Crop duration (days)		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	248.33	162.33	205.33	86.67	87.33	87.00
50	305.00	171.33	238.67	81.67	87.00	84.33
100	265.00	197.00	231.00	81.67	92.33	87.00
150	285.00	176.33	230.67	87.33	92.00	89.67
200	298.33	185.00	241.67	83.00	86.67	84.83
250	260.00	179.33	219.67	81.67	92.00	86.83
300	235.00	163.00	199.00	80.33	92.00	86.17
350	233.33	151.33	192.33	78.67	97.33	88.00
400	228.33	152.67	190.50	82.00	95.33	88.67
Mean	262.04	170.25	216.81	82.55	91.33	86.94
Between treatments CD (0.01)			25.16			NS
Interaction CD (0.01)			35.58			7.25

4.2.2.9 Crop duration

For crop duration, the analysis of variance showed significance difference between the treatments within the species (Table 9). In general, with increase in doses of gamma rays, there was increase in crop duration. In *A. hypochondriacus*, the treatments 50 Gy and 200 Gy were on par with control with a crop duration of 87 days. Maximum duration of 97 days was observed in this species at 350 Gy dose. The effect of 100 Gy, 150 Gy, 250 Gy, 300 Gy, 350 Gy and 400 Gy were on par. In *A. tricolor* (Arun), the effect of all the treatments except 350 Gy were on par with control. Generally, *A. tricolor* (Arun) was having less crop duration than *A. hypochondriacus*.

4.2.2.10 Morphological abnormalities

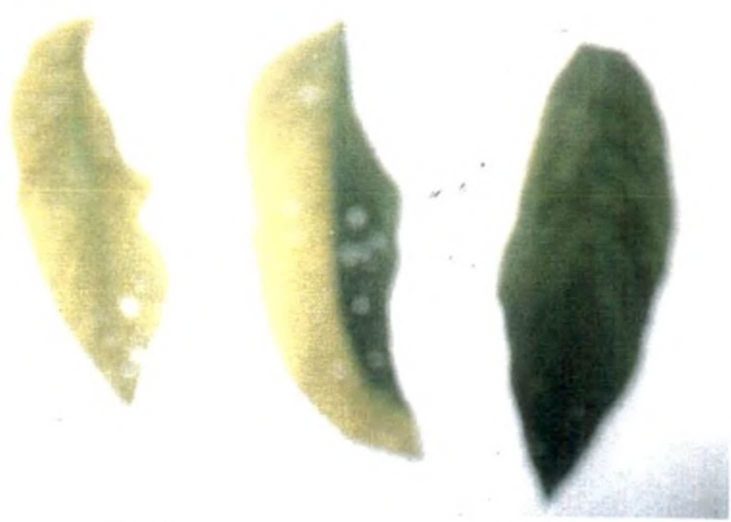
Morphological variations were observed in the M_1 generation of both the species. In *A. tricolor* (Arun), spreading and semi dwarf plants (Plate 3) were observed in the doses of 250 Gy and 200 Gy. These plants had small and round leaves. Branching was observed in the case of *A. hypochondriacus* at a dose of 200 Gy (Plate 4).

Chlorophyll chimeras were noticed in *A. hypochondriacus* (Plate 2).

4.2.3 Effect on disease resistance

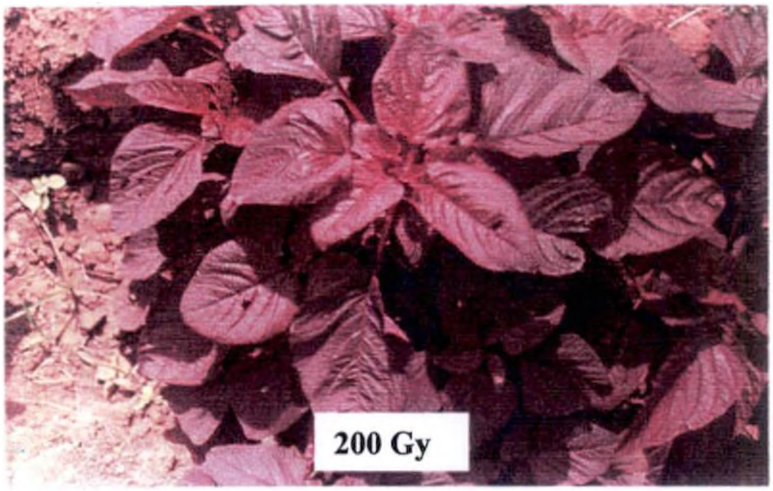
4.2.3.1 Disease incidence

There was no significant variation for disease incidence among the treatments and within the species in M_1 generation (Table 10). The two Amaranth species differed among themselves for disease incidence. *A. hypochondriacus* was completely free from leaf spot disease incidence and the maximum percentage of disease incidence noticed in *A. tricolor* (Arun) was 33 per cent at 250 Gy. Lowest values of disease incidence (18%) was noticed at 50 and 100 Gy dose which is lower than control.



250 Gy Control

Plate 2. Chlorophyll mutants of *A. hypochondriacus* observed in M_1 generation



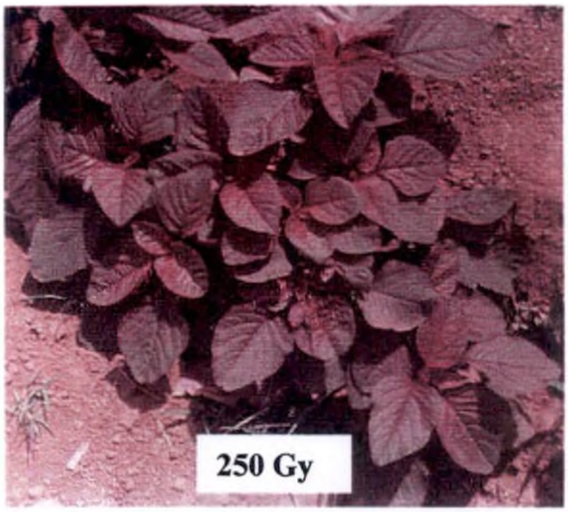


Plate 3. Variability in growth habit observed in the M_1 generation of gamma ray treated plant in *A. tricolor* (Arun)

Plate 4. Variability in growth habit observed in the M_1 generation of gamma ray treated plant in *A. hypochondriacus*

4.2.3.2 Disease severity

The treatments showed no significant difference among themselves and within the species. The species differed among themselves for disease severity. Leaf spot disease was observed only in *A. tricolor* (Arun) whereas *A. hypochondriacus* was completely free from the disease (Table 10). Disease severity was lower in the treatments 50 Gy (10%) and 100 Gy (11%) than control (13%) and maximum in 350 Gy dose (15 %).

4.2.4 Effect on biochemical attributes

4.2.4.1 Beta carotene

Lower doses showed significant variation from the rest of the treatments for Beta carotene content. The mean content of Beta carotene was the highest at 50 Gy in both the species followed by control. *A. tricolor* (Arun) recorded maximum values for Beta carotene content as high as 7561 I.U at 50 Gy followed by control (3808 I.U). In all the doses above 50 Gy the content was significantly lower than control. In *A. hypochondriacus*, the highest content of 3691 I.U was recorded at 50 Gy, which was on par with control (3442 I.U) and other treatments. The lowest value of Beta carotene (2261.95 I.U) was recorded in the treatment 400 Gy (Table 11).

4.2.4.2 Vitamin C

There was significant difference for vitamin C content between the treatments and within the species. The mean content of vitamin C was less in all the treatments than control and was found to decrease with increase in dose as was revealed in Table 11. Between species, *A. hypochondriacus* recorded higher content of vitamin C in all the treatments with maximum value of 203 mg/100 g at 100 Gy dose which was higher than control (169 mg/100 g). The content of vitamin C was less in all the irradiated treatments in *A. tricolor* (Arun) with a maximum value of 180 mg/100 g in control.

Table 10. Effect of gamma rays on disease severity in M_1 generation

Dose Gy	Disease severity				Disease incidence			
	A. tricolor (Arun)		*A. hypochondriacus*		*A. tricolor* (Arun)		*A. hypochondriacus*	
	Disease severity (%)	Range of score	Disease severity (%)	Range of score	Disease incidence(%)	Range of score	Disease incidence (%)	Range of score
0	12.87	3.00	0	1.00	28.50	3.00	0	1.00
50	10.67	3.00	0	1.00	18.97	2.00	0	1.00
100	11.33	3.00	0	1.00	18.97	2.00	0	1.00
150	13.10	3.00	0	1.00	28.50	3.00	0	1.00
200	14.20	3.00	0	1.00	23.73	3.00	0	1.00
250	13.00	3.00	0	1.00	33.27	3.00	0	1.00
300	12.00	3.00	0	1.00	28.50	3.00	0	1.00
350	14.67	3.00	0	1.00	28.50	3.00	0	1.00
400	14.00	3.00	0	1.00	28.50	3.00	0	1.00
Between treatments			NS					NS
Interactions			NS					NS

Table 11. Effect of gamma rays on biochemical attributes in M_1 generation

Dose (Gy)	Beta carotene (I.U.)			Vitamin C (mg/100 g)			Oxalate (%)			Nitrate (%)		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	3808.72	3442.82	3625.77	180.8	169.00	174.90	1.30	1.87	1.58	3.09	1.33	2.21
50	7561.97	3691.18	5626.57	138.93	163.20	151.27	0.96	1.19	1.07	2.88	1.08	1.98
100	2544.67	2483.71	2514.19	109.53	203.00	156.26	1.09	1.56	1.32	3.03	1.21	2.12
150	2727.63	3060.27	2893.95	96.90	145.20	121.05	1.63	1.39	1.51	3.11	1.63	2.37
200	2805.20	2877.31	2841.26	89.93	145.20	117.56	1.47	1.63	1.55	3.13	1.98	2.55
250	2311.83	2905.04	2608.44	83.46	133.00	108.23	1.70	2.30	2.00	3.40	1.95	2.67
300	2317.02	2622.31	2469.66	83.46	108.97	96.21	1.90	2.43	2.17	3.40	2.10	2.75
350	2300.70	2411.64	2356.17	55.23	108.97	82.10	2.17	2.90	2.53	3.50	2.33	2.92
400	2167.69	2261.95	2214.82	41.80	108.97	75.38	2.40	3.23	2.82	3.53	2.43	2.98
Mean	3171.71	2861.80	3016.75	97.78	142.83	120.32	1.62	2.05	1.83	3.23	1.78	2.50
Between treatments CD (0.01)			1231.14			18.30			0.48			0.24
Interaction CD (0.01)			1741.10			25.90			NS			0.33

4.2.4.3 Oxalate

The treatments differed significantly for oxalate content irrespective of the species. In general, higher doses showed more oxalate content as compared to lower doses. The oxalate content was lower than control at 50 Gy with a value of 1.07 per cent (Table 11). The untreated plants had an oxalate content of 1.58 per cent The oxalate content had values ranging from 0.96 per cent to 2.4 per cent in *A. tricolor* (Arun) and 1.19 per cent to 3.23 per cent in *A. hypochondriacus*.

4.2.4.4 Nitrate

The result on nitrate content is presented in Table 11. There was significant difference between the treatments and within the species. Here also, the mean content of nitrate was lower than control in 50 Gy dose and it increased as the dose increased. Among the species, *A. tricolor* (Arun) recorded higher values for nitrate content than *A. hypochondriacus*. In *A. tricolor*, the nitrate content ranged from 2.88 per cent in 50 Gy to 3.53 per cent in 400 Gy. In *A. hypochondriacus*, lower content of nitrate was observed at 50 Gy dose than control with a value of around 1 per cent.

4.3 EFFECT OF GAMMA RAYS IN M_2 GENERATION

4.3.1 Effect of gamma rays on seed germination and survival under field conditions

a) Germination percentage

A reduction in germination percentage corresponding to increase in doses of gamma rays was noticed in M_2 generation. There was significant difference in germination percentage between the treatments irrespective of the species. The untreated seeds showed maximum germination percentage of 78.33 followed by the treatments 50 Gy, 100 Gy, 150 Gy, 250 Gy and 300 Gy which were on par with each other and had germination between 65-70 per cent. With doses above 300 Gy there was significant decline in germination percentage. Minimum germination was observed for 350 Gy and 400 Gy, which had values around 60 per cent. Significant variation for germination percentage between *A. tricolor* (Arun) and

Table 12. Effect of gamma rays on seed germination in M_2 generation

Doses (Gy)	*A. tricolor* (Arun)		*A. hypochondriacus*		Mean
	Germination (%)	Relative percent over control	Germination (%)	Relative percent over control	
0	77.33	100.00	79.33	100.00	78.33
50	68.33	88.36	71.00	89.49	69.67
100	67.67	87.50	73.33	92.43	70.50
150	65.67	84.92	76.00	95.80	70.83
200	58.33	75.42	68.00	85.71	63.17
250	64.33	83.18	65.33	82.35	64.83
300	65.33	84.48	67.33	84.87	66.33
350	57.67	74.57	63.67	80.25	60.67
400	56.33	72.84	63.00	79.41	59.67
Mean	64.55		69.66		67.10
Between treatments	CD (0.01)				6.59
Interaction					NS

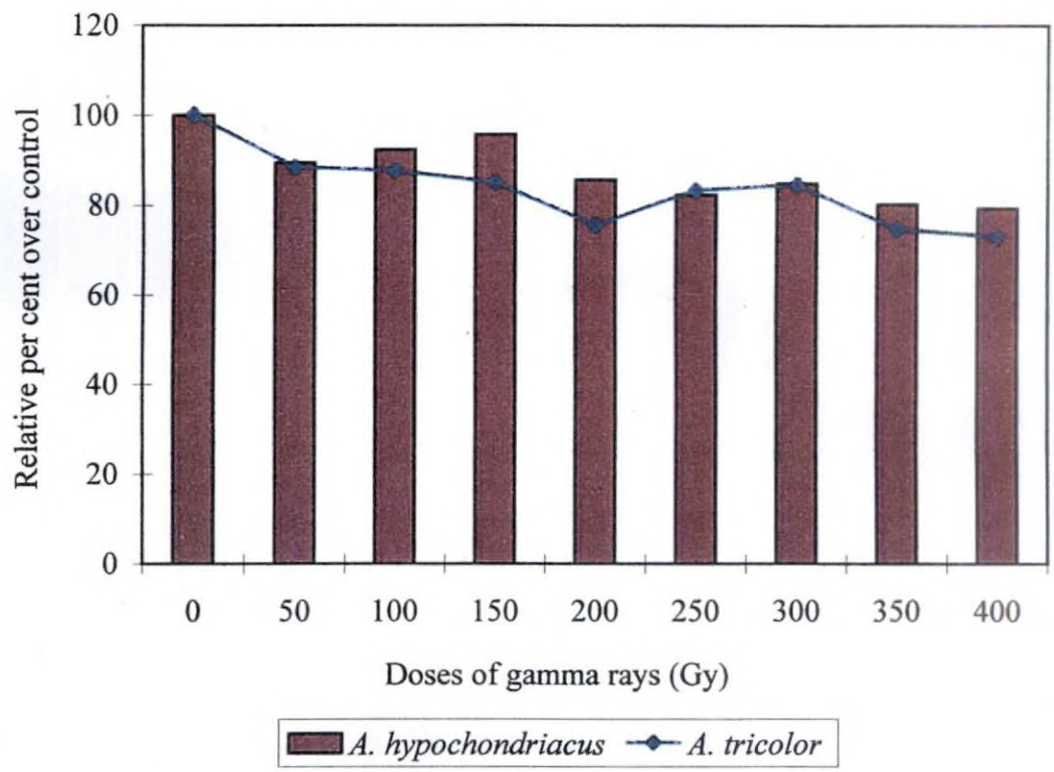


Fig. 5. Effect of gamma rays on seed germination in M_2 generation

A. hypochondriacus was noticed. Maximum germination percentage of 79.33 was observed in untreated populations of *A. hypochondriacus* and minimum for 400 Gy dose of *A. tricolor* (Arun) with germination of 56.33 per cent as can be observed from Table 12 and Fig.5.

b) Survival percentage

The analysis of variance for survival percentage (Table 13) revealed a significant difference between treatments irrespective of species. Again, maximum survival percentage was observed for untreated populations (74.33%) followed by decrease in survival percentage with increase in doses of gamma rays. Survival percentage at 50, 100 and 150 Gy were on par and was 65-66 per cent. Doses above 150 Gy showed a reduction in survival percentage and was minimum for 400 Gy with a value of 48.50 per cent. The survival percentage is graphically represented in Fig.6.

4.3.2 Effect of gamma rays on morphological characters

4.3.2.1 Plant height at first harvest

The data on plant height at first harvest in Table 14 revealed that the treatments differed significantly for plant height irrespective of the species. Doses ranging from 50 Gy to 350 Gy showed a stimulatory effect on plant height and were on par with each other with values ranging from 65.3 cm in 300 Gy to 78.3 cm in 250 Gy. Plant height was lower at 400 Gy dose (58.7 cm) and it was on par with control (63.42 cm). *A. tricolor* (Arun) differed significantly with *A. hypochondriacus* for plant height. It ranged from 49.59 cm in *A. tricolor* (Arun) to 88.64 cm in *A. hypochondriacus*.

4.3.2.2 Plant height at flowering

Mean plant height at flowering for both the species was on par with control (91 cm) in all the doses except 400 Gy (80 cm) (Table 14). *A. hypochondriacus* (114 cm) was significantly taller than *A. tricolor* (Arun) (72 cm) in all the treatments. In *A. hypochondriacus*, highest plant height was seen in 250 Gy (134.33 cm) followed

Table 13. Effect of gamma rays on survival in M_2 generation

Doses (Gy)	*A. tricolor* (Arun)		*A. hypochondriacus*		Mean
	Survival (%)	Relative percent over control	Survival (%)	Relative percent over control	
0	74.33	100.00	74.33	100.00	74.33
50	65.00	87.44	66.33	89.23	65.67
100	65.00	87.44	67.33	90.58	66.17
150	62.00	83.41	68.00	91.48	65.00
200	55.00	73.99	62.00	83.41	58.50
250	60.00	80.72	57.00	76.68	58.50
300	60.67	81.62	61.00	82.06	60.83
350	53.33	71.74	54.67	73.55	54.00
400	46.67	62.78	50.33	67.71	48.50
Mean	60.22		62.33	61.27	
Between treatments	CD(0.01)				6.46
Interaction					NS

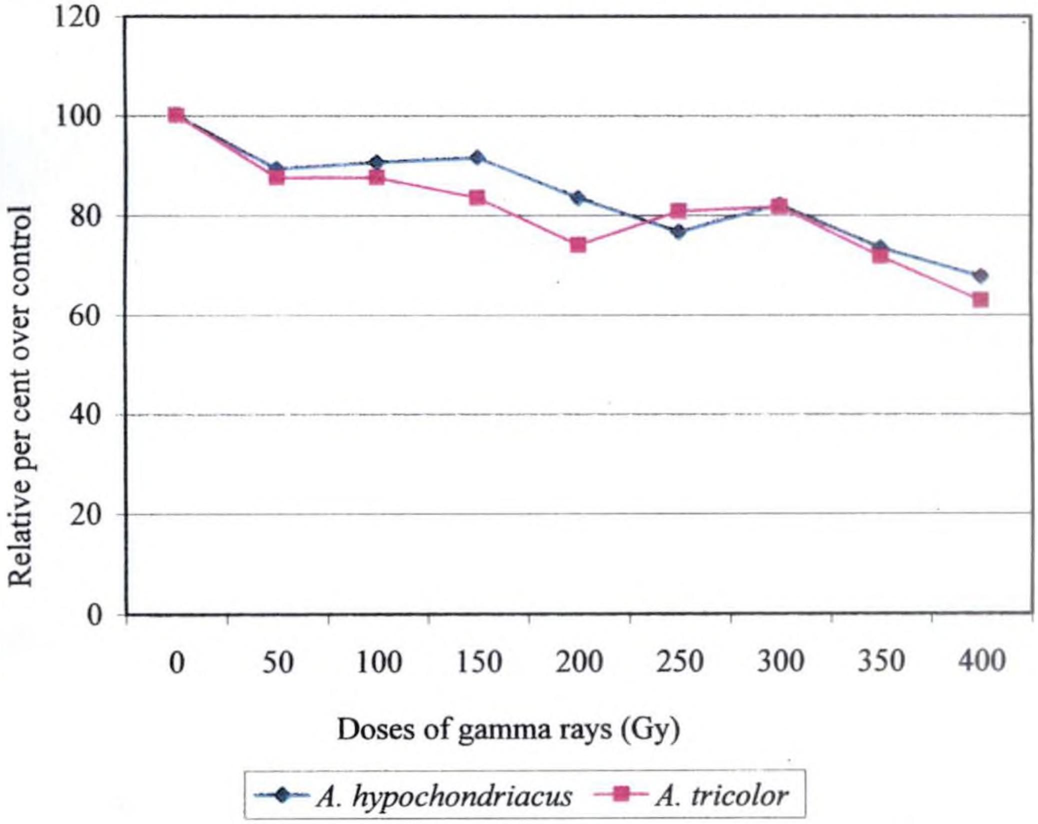


Fig. 6. Effect of gamma rays on survival in M_2 generation

Table 14. Effect of gamma rays on plant height in M_2 generation

Dose (Gy)	Plant height at harvest (cm)			Plant height at flowering (cm)		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	48.97	77.87	63.42	71.27	111.33	91.30
50	57.40	89.80	73.60	78.00	116.67	97.33
100	55.67	86.67	71.76	74.40	109.67	92.03
150	49.47	89.00	69.23	73.67	110.00	91.83
200	48.73	104.80	76.77	70.73	132.67	101.70
250	50.20	106.40	78.30	72.87	134.33	103.60
300	46.73	83.93	65.33	72.00	119.60	95.80
350	47.87	83.13	65.50	72.23	105.53	88.88
400	41.27	76.20	58.73	69.67	91.13	80.40
Mean	49.59	88.64	69.18	72.74	114.49	93.65

Between treatments CD (0.01)			13.61			14.90
Interaction CD (0.01)			NS			21.10

by 200 Gy which was higher than the untreated populations. In *A. tricolor* (Arun) plant height at flowering ranged from 69.67 cm in 400 Gy dose to 78 cm in 50 Gy dose.

4.3.2.3 Branches per plant

Branches per plant were significantly different between the treatments irrespective of the species (Table 16). The mean number of branches per plant was found to be higher and on par with control in lower doses. At higher doses above 250 Gy, the number of branches per plant was found to be below control. Number of branches per plant was significantly different between the two species. Mean number of branches was found to be more in *A. tricolor* (Arun) with a value of 12.94 and less in *A. hypochondriacus* with a value of 7.94.

4.3.2.4 Leaf length

The data on leaf length presented in Table 15 revealed that there was significant difference between the treatments irrespective of the species. In case of lower doses of 50 Gy and 100 Gy, mean leaf length was 19.08 cm and 20.15 cm respectively which were much higher than the untreated population showing a mean leaf length of 18.3 cm. Rest of the treatments were on par with control and had leaf length varying from 17.42 cm in 400 Gy dose to 18.71 cm in 200 Gy dose. Leaf length ranged from 17.57 cm in 300 Gy to 18.52 cm in 100 Gy of *A. tricolor* (Arun) while it was varying from 17.18 cm in 400 Gy dose to 21.77 cm in 100 Gy dose of *A. hypochondriacus*.

4.3.2.5 Leaf width

The analysis of variance showed no significant difference between the treatments for leaf width. The treatments did not differ significantly within the species also. But leaf width was varying significantly between the two species. Leaf width was more in *A. tricolor* (Arun) (10.5 cm) than *A. hypochondriacus* (7.9 cm) (Table 15).

Table 15. Effect of gamma rays on branches per plant, leaf length and leaf width in M_2 generation

Dose (Gy)	Branches per plant			Leaf length (cm)			Leaf width (cm)		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	13.33	7.93	10.63	17.68	18.92	18.30	10.72	8.09	9.403
50	14.33	8.47	11.40	17.83	20.33	19.08	10.90	8.23	9.56
100	13.26	8.33	10.80	18.52	21.77	20.15	11.09	8.27	9.68
150	14.73	8.47	11.60	17.90	19.05	18.47	10.67	8.52	9.59
200	13.53	7.60	10.57	17.73	19.68	18.71	10.98	8.09	9.53
250	15.27	8.50	11.88	17.71	19.59	18.65	10.37	8.15	9.26
300	9.62	7.53	8.57	17.57	19.01	18.29	10.24	7.92	9.08
350	11.30	7.73	9.52	17.58	18.27	17.93	9.78	7.35	8.57
400	11.12	6.87	8.99	17.66	17.18	17.42	9.91	7.31	8.61
Mean	12.94	7.94	10.44	17.78	19.31	18.55	10.52	7.99	9.25
Between treatments	CD (0.01)		2.14	CD (0.05)		1.39	CD (0.05)		NS
Interaction	CD (0.01)		NS	CD (0.05)		NS	CD (0.05)		NS

4.3.2.6 Leaf stem ratio

The treatments differed among themselves for leaf stem ratio irrespective of the species (Table 15). Mean leaf stem ratio was the highest for 100 Gy (1.25), which was on par with control and all treatments except 50 Gy (1.02), 300 Gy (0.91) and 400 Gy (0.98). Leaf stem ratio ranged from 0.98 in 300 Gy dose to 1.58 in 100 Gy dose in *A. tricolor* (Arun) while it varied from 0.82 in control to 0.96 in 250 Gy dose in *A. hypochondriacus*

4.3.2.7 Days to 50 per cent bolting

The treatments differed significantly for days to bolting among themselves and within the species (Table 16). Early bolting of 73 days was noticed at 100 Gy whereas bolting was very late in all other treatments with a mean number of days of 78-81. In *A. hypochondriacus*, plants at 100 Gy dose took only 67 days to flower whereas in *A. tricolor* (Arun), all the doses were on par with control (77-82 days).

4.3.2.8 Vegetable yield (g/plant)

The results presented in Table 17 revealed that there was significant difference among the different treatments for vegetable yield per plant. In general, vegetable yield was decreasing with increasing dose rates of gamma rays. The mean vegetable yield was significantly higher than control (207 g/plant) in doses of 50 Gy, 100 Gy, 200 Gy and 250 Gy with values ranging from 226 g to 247 g. Lowest yield of 191 g was noticed at 400 Gy dose. Significant differences for treatments within the species and between species were also noticed. Vegetable yield was maximum at 50 Gy dose (278 g/plant) and minimum at 350 Gy dose (228 g/plant) in *A. tricolor* (Arun) while in *A. hypochondriacus*, yield was maximum at a dose of 250 Gy (246 g/plant) followed by 200 Gy (239 g/plant) and minimum at 400 Gy dose (151 g/plant).

4.3.2.9 Crop duration

The analysis of variance showed that crop duration differed significantly between treatments and within the species (Table 17). The mean crop duration was

Table 16. Effect of gamma rays on leaf stem ratio and days to bolting in M_2 generation

Dose (Gy)	Leaf stem ratio			Days to 50 per cent bolting		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	1.36	0.82	1.09	77.67	80.00	78.83
50	1.22	0.82	1.02	79.33	81.33	80.33
100	1.58	0.92	1.25	80.67	67.00	73.83
150	1.37	0.88	1.12	81.00	81.33	81.17
200	1.37	0.83	1.09	77.00	81.33	79.17
250	1.28	0.96	1.12	78.33	80.00	79.17
300	0.98	0.83	0.91	82.00	81.33	81.67
350	1.25	0.88	1.06	78.33	80.00	79.17
400	1.13	0.83	0.98	81.33	78.67	80.00
Mean	1.28	0.86	1.07	79.52	75.26	79.26
Between treatments		CD (0.05)	0.17	CD (0.01)		5.178
Interaction		CD (0.01)	NS	CD (0.01)		7.32

Table 17. Effect of gamma rays on vegetable yield and crop duration in M_2 generation

Dose (Gy)	Vegetable yield (g/plant)			Crop duration (days)		
	A. tricolor (Arun)	*A. hypochondriacus*	Mean	*A. tricolor* (Arun)	*A. hypochondriacus*	Mean
0	238.50	176.88	207.69	108.33	115.33	111.83
50	278.44	189.00	233.72	109.33	115.33	112.33
100	264.00	188.55	226.27	110.67	98.33	104.50
150	259.55	153.83	206.69	111.00	117.00	114.00
200	250.55	239.77	245.16	111.33	115.33	113.00
250	247.33	246.88	247.10	108.33	114.00	111.17
300	244.44	182.88	213.66	112.00	115.33	113.67
350	228.77	164.33	196.55	108.33	111.33	109.83
400	231.55	151.33	191.44	107.00	110.00	108.50
Mean	249.24	188.16	218.69	109.59	112.40	110.99
Between treatments		CD (0.01)	32.72			4.85
Interaction		CD (0.01)	47.68			6.86

maximum at 150 Gy dose with 114 days and minimum at 100 Gy dose with 104 days. In *A. tricolor* (Arun), all treatments were on par with control and it was ranging from 107 days to 112 days. Maximum crop duration of 117 days was recorded at a dose of 150 Gy in *A. hypochondriacus* and a minimum of 98 days at 100 Gy.

4.3.3 Effect on leaf spot resistance

4.3.3.1 Disease incidence

There was no significant difference for disease incidence between the treatments and within the species. All the treatments in *A. tricolor* (Arun) showed 100 per cent disease incidence at all stages of scoring. Not a single plant in this species was free from disease. *A. hypochondriacus* was immune to leaf spot infection.

4.3.3.2 Disease severity

Disease severity was taken at 15th, 30th and 45th day after transplanting. At 15th day after transplanting, maximum disease severity of 30.4 per cent was recorded for the untreated population and least for 50 Gy, 100 Gy and 150 Gy dose with a disease severity of around 19 per cent. The observations on 30th day after transplanting was used for deciding the category to which the treatments belonged since this period was the peak period for leaf spot infection and the data is presented in Table 18. The results presented on disease severity at 30th day after transplanting revealed significant difference between the treatments within the species. Disease severity was maximum for control and 400 Gy dose with a value around 50 per cent followed by 250 Gy dose (43%) while the least disease severity of 25 per cent was in the case of 100 Gy dose (Plate 7). *A. hypochondriacus* was completely resistant to leaf spot infection in all the stages. There was no significant difference between the treatments within the species for disease severity at 45th day after transplanting in *A. tricolor*. Based on disease severity 100 Gy was classified as moderately resistant to leaf spot while control and 400 Gy was identified as highly susceptible group.

Table 18. Effect of gamma rays on leaf spot infection in M_2 generation at 15^{th}, 30^{th} and 45^{th} day after transplanting

Dose (Gy)	15th day after transplanting				30th day after transplaning						45th day after transplanting			
	A. tricolor (Arun)		*A. hypochondriacus*		*A. tricolor* (Arun)			*A. hypochondriacus*			*A. tricolor* (Arun)		*A. hypochondriacus*	
	Disease severity (%)	Score	Disease severity (%)	Score	Disease severity (%)	Score	React-ion	Disease severity (%)	Score	React-ion	Disease severity (%)	Score	Disease severity (%)	Score
0	30.4	4.00	0	1	50.40	5	H.S.	0	1	Immune	22.66	3.00	0	1
50	18.40	3.00	0	1	33.60	4	M.S	0	1	,,	11.66	3.00	0	1
100	18.93	3.00	0	1	24.8	3	M.R	0	1	,,	11.53	3.00	0	1
150	19.20	3.00	0	1	33.07	4	M.S	0	1	,,	13.86	3.00	0	1
200	22.67	3.00	0	1	33.07	4	M.S	0	1	,,	14.60	3.00	0	1
250	26.67	4.00	0	1	42.93	4	M.S	0	1	,,	24.8	3.00	0	1
300	24.8	3.00	0	1	41.60	4	M.S	0	1	,,	20.06	3.00	0	1
350	27.47	4.00	0	1	39.20	4	M.S	0	1	,,	18.66	3.00	0	1
400	27.47	400	0	1	50.13	5	H.S	0.	1	,,	28.53	4.00	0	1

Between treatments CD (0.01) 0.32 CD (0.05) 0.44 NS
Interaction CD (0.01) 0.45 CD (0.05) 0.63 NS

4.3.4 Effect on biochemical attributes

4.3.4.1 Beta carotene

The treatments showed significant difference for Beta carotene content among themselves and within the species (Table 19). Maximum mean Beta carotene content of 2871.77 I.U. was recorded in 150 Gy dose which was on par with control and all doses except 200 Gy, 250 Gy and 400 Gy. In *A. tricolor* (Arun), higher content of Beta carotene was observed for lower dose. Highest value was recorded in 50 Gy dose which was on par with control, 100 Gy, 150 Gy and 300 Gy dose. Minimum content of Beta carotene was observed in 200 Gy and 400 Gy doses (1700 I.U.). In *A. hypochondriacus* Beta carotene content was found to be significantly higher than control (2921 I.U.) in lower doses of 100 Gy (3426.18 I.U.), 150 Gy (3758.82 I.U.) and 300 Gy (3315.31 I.U.).

4.3.4.2 Vitamin C

The mean content of vitamin C was significantly different between the treatments (Table 19). Maximum mean vitamin C content of 146.28 mg/100 g was observed in 50 Gy dose which was on par with 100 Gy and 150 Gy while the highest dose of 400 Gy recorded the least vitamin C content (96.71 mg/100 g) which was on par with control. In *A. hypochondriacus*, maximum vitamin C content of 138.36 mg/100 g was observed for 50 Gy dose which was on par with 100 Gy to 200 Gy dose and control and from there onwards, it showed a decreasing trend. *A. tricolor* (Arun) recorded higher content of vitamin C in lower doses with a maximum value of 154 mg at 50 Gy and 100 Gy doses which was higher than control (105 mg/100 g).

4.3.4.3 Oxalate

Generally, a progressive increase in mean oxalate content with increase in dosage rates of gamma rays was observed (Table 19). Least mean oxalate content was noted for control (1.67%) which was on par with 50 Gy and 100 Gy doses. Oxalate content ranged from 2.03 per cent in control to a maximum of 2.83 per cent in 200 Gy dose in case of *A. hypochondriacus* but all doses were on par statistically. In *A. tricolor* (Arun), least oxalate content of 1.3 per cent was observed in control, which

Table 19. Effect of gamma rays on biochemical attributes in M_2 generation

Dose (Gy)	Beta carotene (I.U)			Vitamin C (mg/100 g)			Oxalate (%)			Nitrate (%)		
	A tricolor (Arun)	*A. hypochondriacus*	Mean	*A tricolor* (Arun)	*A. hypochondriacus*	Mean	*A tricolor* (Arun)	*A. hypochondriacus*	Mean	*A tricolor* (Arun)	*A. hypochondriacus*	Mean
0	2392.49	2921.68	2657.08	105.87	115.89	110.88	1.30	2.03	1.67	1.37	2.47	1.92
50	2694.38	2761.13	2727.75	154.19	138.36	146.28	1.56	2.16	1.86	1.15	2.23	1.69
100	2128.88	3426.18	2777.53	154.19	115.32	134.76	1.63	2.35	1.99	1.37	2.33	1.85
150	1984.72	3758.82	2871.77	137.30	121.31	129.30	2.27	2.52	2.39	2.81	2.87	2.84
200	1702.00	2755.36	2228.68	105.87	121.31	113.59	2.14	2.83	2.49	3.13	2.70	2.92
250	1884.96	2666.52	2275.73	99.91	110.43	105.17	2.43	2.37	2.39	3.12	2.77	2.99
300	1957.03	3315.31	2636.17	88.98	110.43	99.70	3.33	2.77	3.05	3.32	3.27	3.29
350	1890.50	2927.23	2408.86	88.98	110.43	99.70	3.33	2.53	2.93	3.69	3.80	3.75
400	1727.48	2516.97	2122.22	83.00	110.43	96.71	3.20	2.20	2.70	3.69	3.83	3.76
Mean	2295.31	3381.15	2838.22	127.29	145.54	129.51	2.65	2.75	2.47	2.96	3.28	3.13
Between treatment	CD (0.01)		553.63	CD (0.01)		26.39	CD (0.01)		0.76		CD(0.05)	0.29
Interaction	CD (0.01)		782.96	CD (0.05)		27.66	CD (0.01)		1.07		CD(0.05)	0.42

was on par with 50 to 200Gy doses. Maximum oxalate content of 3.3 per cent was observed at 300 Gy to 400 Gy doses.

4.3.4.4 Nitrate

The treatments differed significantly among themselves and with the species as could be observed from Table 19. Mean nitrate content was significantly lower at lower doses (50 Gy and 100 Gy) which was on par with control. Nitrate content showed an increasing trend with increase in doses of gamma rays in both the species. In *A. tricolor* (Arun), maximum value of 3.69 per cent was recorded for 350 Gy and 400 Gy dose and least in 50 Gy (1.15%), which was on par with control and 100 Gy dose. In *A. hypochondriacus*, a slight decrease in nitrate content could be observed than control at lower doses of 50 Gy and 100 Gy (2.3%). Maximum nitrate content of 3.8 per cent was recorded for 350 Gy and 400 Gy doses.

4.3.5 Viable mutations or macromutations

The spectrum of viable mutations was much wider in *A. hypochondriacus* than *A. tricolor* (Arun). In *A. hypochondriacus*, the spectrum of viable mutations was wider with gamma ray treatments at 100 Gy dose(9.26) followed by 250 Gy (4.66) and 350 Gy (1.99) (Table 20). All the doses except 50 Gy and 200 Gy were effective to induce viable macromutations. More branching, dwarf plants, early flowering, fasciation, reduced leaf size, presence of leaflets and inflorescence mutant were observed randomly in certain doses of gamma rays (Plate 5, Plate 6). In *A. tricolor* (Arun), the frequency and spectrum of viable mutations was lesser and narrower than *A. hypochondriacus*. Mutation frequency was higher in higher dose of 250 Gy (5.02) followed by 100 Gy dose (2.56) in this species (Table 21). The spectrum of viable mutations included high yielding mutants, branched mutants and leaf mutants.

Among the viable mutants, 8 economic mutants were identified based on desired characters viz., dwarfness, early flowering in grain types, branching and high vegetable or seed yield (Plate 8). The salient features of the eight mutants are presented in Table 22 and Table 23.

Table 20. Spectrum of viable mutations and their frequency per 100 M_2 plants of *A. hypochondriacus*

Mutagenic treatment	Total plant population	Plant type mutants			Maturity mutants	Leaf mutants	Inflorescence mutants	High seed yielding mutants	Mutation frequency per 100 M_2 plants
		Branching nature	Fasciation	Dwarf	Early flowering				
50	150	-	-	-	-	-	-	-	-
100	150	0.66 (1)	-	2.67 (4)	2.67 (4)	1.30 (2)	0.66 (1)	1.30 (2)	9.26
150	135	0.74 (1)	-	-	-	-	-	-	0.74
200	150	-	-	-	-	-	-	-	-
250	150	2.67 (4)	1.33 (2)	-	-	0.66 (1)	-	-	4.66
300	120	1.67 (2)	-	-	-	-	-	-	1.67
350	150	1.33 (2)	0.66 (1)	-	-	-	-	-	1.99
400	135	0.74 (1)	-	-	-	-	-	-	0.74

Figures in parenthesis denote number of mutants

Table 21. Spectrum of viable mutations and their frequency per 100 M_2 plants of *A. tricolor* (Arun)

Mutagenic treatments	Total plant population	Plant type			Maturity mutants	Leaf mutants		High vegetable yielding mutants	Mutation frequency per 100 M_2 plants
		Branching	Dwarf	Fasciation		Leaf shape	Colour		
50	156	-	-	-	-	-	-	-	-
100	156	1.28(2)	-	-	-	-	-	1.28(2)	2.56
150	156	-	-	-	-	-	-	-	-
200	156	0.64(1)	-	-	-	-	-	0.64(1)	1.28
250	156	0.64(1)	-	-	-	0.64(1)	3.10(5)	0.64(1)	5.02
300	156	-	-	-	-	-	-	-	-
350	156	-	-	-	-	-	-	-	-
400	156	-	-	-	-	-	-	-	-

Figures in parenthesis denote number of mutants

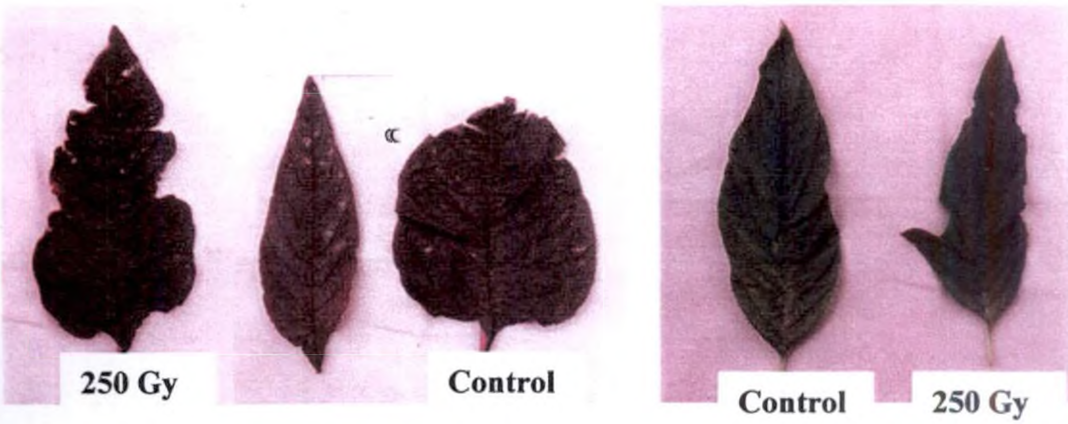


Plate 5. Variations in leaf shape and colour in M_2 generation of *Amaranthus spp.*

Branching mutant

Fasciation

Plate 6. Variation in growth habit in the M_2 generation of *A. hypochondriacus*

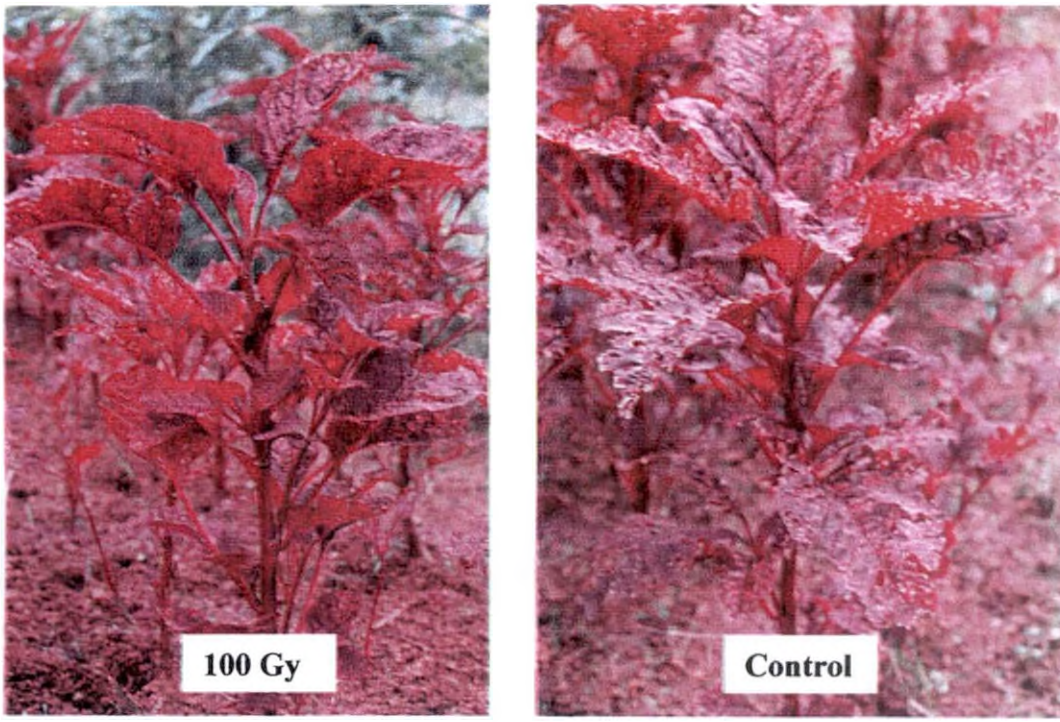
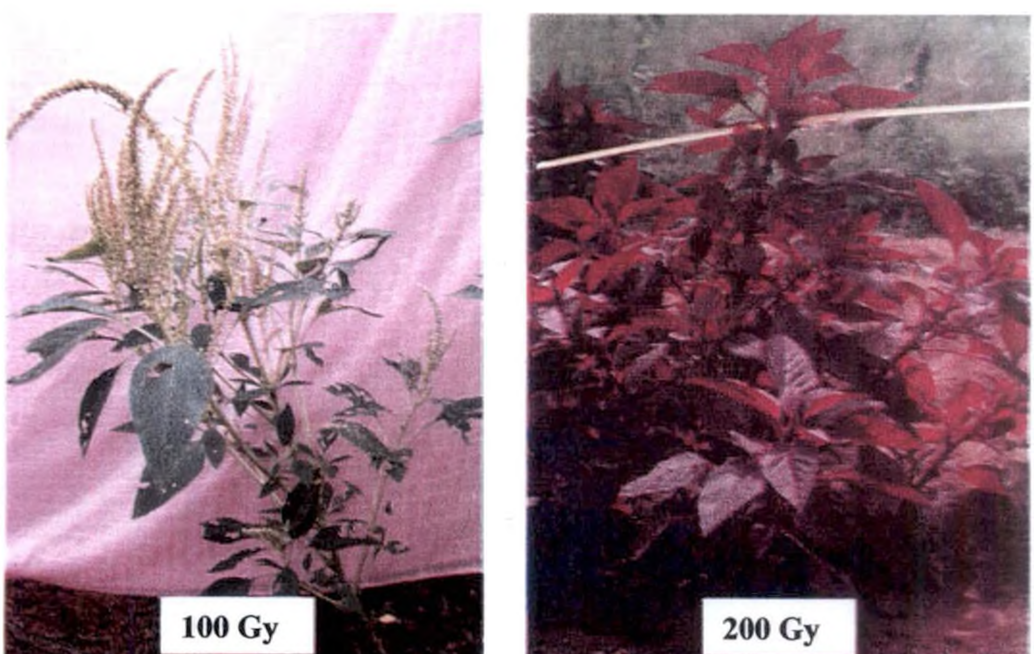


Plate 7. Variability in leaf spot infection



Dwarf, early and inflorescence mutant

High yielding mutant

Plate 8. Economic mutants observed in M_2 generation

Table 22. Salient features of economic mutants in *A. hypochondriacus*

Mutant No.	Doses of gamma rays (Gy)	Branches per plant	Leaf length (cm)	Leaf width (cm)	Days to flowering	Height at flowering (cm)	Inflorescence	Seed yield (g)	Crop duration (days)
Mutant 1	100	8	8.5	4.5	35	65	Inflorescence with lateral branching	48	85
Mutant 2	100	7	13.0	6.5	40	72	Panicle	42	95
Mutant 3	100	7	13.5	8.5	40	80	Panicle	35	95
Mutant 4	100	6	14.0	7.0	45	83	Panicle	40	95

Table 23. Salient features of economic mutants in *A. tricolor* (Arun)

Mutant No.	Doses of gamma rays (Gy)	Branches per plant	Leaf length (cm)	Leaf width (cm)	Plant height at harvest (cm)	Vegetable yield (g/plant)	Leaf stem ratio
Mutant 5	100	18	18.0	11.5	58.0	420	1.30
Mutant 6	100	17	17.5	11.0	60.0	400	1.00
Mutant 7	200	22	17.5	8.0	65.0	525	0.98
Mutant 8	250	20	17.0	8.0	60.0	500	1.60

Discussion

5. DISCUSSION

Amaranth is an important vegetable cum grain crop grown for its high nutrient plant part. Among the various diseases affecting the crop, leaf spot disease was first observed in India during 1966. But it has become a serious problem in recent years in amaranth growing tracts of Kerala, causing great damage to the crop and thereby reducing yield and quantity to a considerable extent. The causal organism of leaf spot in *A. tricolor* is *Rhizoctonia solani.*

The conventional plant protection measures for the control of disease are inefficient and undesirable from the point of view of human health and environmental hazards. In 1987, large quantities of leafy vegetables exported from Malaysia to Singapore were rejected due to high levels of ethylene dis dithiocarbamate residues in the produce by application of the fungicide mancozeb (Mah *et al.*, 1988).

The effective way to combat the disease is the use of disease resistant varieties. Varieties of amaranth differ in disease resistance. In Kerala, all the *A. tricolor* cultivars including the commonly cultivated red varieties are Kanara Local and Arun are highly susceptible to the disease whereas Co.1 (*A. dubius*) and a few grain types (*A. hypochondriacus*) were found resistant to the disease (Krishnakumary, 2000).

Monoecious species of Amaranthus are chiefly self pollinated (Subramanian *et al.*, 1997). The vegetable amaranth are predominantly self pollinated due to the presence of number of male flowers per glomerule, small and non showy terminal inflorescence and also due to greater development of axillary glomerules and hence the existing variability in amaranth is limited (Pal and Khoshoo, 1973). In grain amaranth species since the inflorescences are coloured, they are occasionally visited by bees and by this cross pollination pollination is encouraged. Earlier studies revealed that hybridization studies between different species and varieties resulted in high sterility in interspecific hybrids of amaranth (Devdas and Mallika, 1991).

Many scientists have reported that radiation is as efficient as hybridization in supplementing genetic variability for selection. Mutation breeding is considered to be one of the most promising techniques in the genetic improvement of a crop. The distinct advantage of breeding through induced mutation is the possibility/probability to bring about a positive change in one or a few characters of an otherwise superior genotype with no detriment to its desirable characters and if these desirable characters are introduced by mutagenesis, it is certainly the simplest means to achieve the breeding objective. Under these circumstances, mutation breeding will be an efficient and alternate method of breeding in amaranth. Basic information on the type and doses of mutation and their relative effectiveness and efficiency are essential before programming for mutation breeding to achieve the specific objective for which the mutagenesis is aimed at. Such basic information in case of Amaranth was very much limited and investigations were carried out to obtain the same and to see the effect of irradiation on leaf spot infection and yield attributes. A brief discussion on the varied response of Amaranth to mutagenic treatment in the M_1 and M_2 generation is given in the following section.

5.1 EFFECT OF MUTAGENS IN M_1 GENERATION.

The induction of mutation by a mutagen is invariably associated with the production of undesirable changes in the biological material. Damage to plants in the M_1 generation resulting from the biological effects in seeds by mutagens could be measured by several criteria such as reduction in germination and survival, reduction in plant growth, reduction in fertility, increase in the frequency of chromosomal aberrations and increase in the frequency of chlorophyll deficient chimeras.

5.1.1 Estimation of lethal dose

5.1.1.1 Germination percentage

In general there was a progressive reduction in the mean values for germination percentage in both laboratory and field conditions. The germination percentage was found to decrease with the increase in the level of irradiation. Percentage reduction in germination in *A. tricolor* (Arun) was found to be 60 per cent at 750 Gy and around 80 per cent at 1000 Gy. In *A.hypochondriacus*, 50 per cent reduction was observed at 750 Gy and 75 per cent at 1000 Gy. These results are in close agreement with the findings of Behera and Patnaik (1979) in amaranth, Asha (1984) in chilli, Oommen and Gopimany (1984) in cowpea, Abraham (1985) and Krishna (1985) in bhindi, Sayed (1986) in fenugreek, Mohideen (1988) in amaranth, Mini (1989) in cowpea, Dharan (1993) in winged bean, Animon (1996) in bhindi and Nair (1996) in limabean.

Decrease in germination may be due to lethal combination of mutant genes manifesting the lethal effects at the germination stage itself. Reduction in germination may be attributed to a drop in the auxin level as reported by Gordon (1957). Reduced germination at moderate and high doses can be due to varied response of irradiation on the chromosome compliments or due to adverse physiological effects (Pushkaran, 1983).

5.1.1.2 Survival

There was a linear reduction in survival with an increase in dose in both the amaranth species. Among the treatments in the 2 species, survival percentage was always higher in *A. hypochondriacus* then *A. tricolor* (Arun) upto 500 Gy. At doses higher than this, it was found that *A. hypochondriacus* is highly sensitive to higher doses of gamma rays since no survival of plants could be observed at doses higher than 500 Gy. In *A. tricolor* (Arun), 10 per cent survival was observed at 750 Gy and zero per cent at 1000 Gy. This also indicates the difference between the two species towards the response to physical mutagen. The reduction in survival is an index of post germination mortality in treated plants as a result of cytological and physiological disturbances due to radiation effect. The cytological abnormalities may lead to structural changes in the chromosomes. This interferes with the normal growth and development of organs which might have lead to the reduction in survival percentage with irradiation (Sreekumari Amma, 1985). The results are in agreement with the findings of Raghuvanshi and Singh (1977) in fenugreek, Behera and Patnaik (1979) in amaranth, Oommen and Gopimany (1984) in cowpea, Abraham (1985) and Krishna

(1985) in bhindi, Sayed (1986) in fenugreek, Mohideen (1988) in amaranth, Mini (1989) in cowpea, Animon (1996) in bhindi and Nair (1996) in limabean.

5.1.2 Plant Height

In M_1 generation the means values for plant height at harvest and at flowering showed significant decrease with increase in doses of gamma rays in both the species. Similar shift in mean values for plant height has been reported by Sayed (1986) in fenugreek, Mohideen (1988) in amaranth, Dharan (1993) in winged bean, Animon (1996) in bhindi, Gunasekharan *et al.* (1996) in cowpea, Nair (1996) in limabean and Mohanasundaram *et al.* (1997) in cowpea. Reduced growth in mutagen treated materials at higher doses were attributed to abnormal cytological behaviour due to chromosomal damage and mitotic inhibition (Sparrow *et al.*, 1952). Mikaelsen (1968) reported inhibition of DNA synthesis as the cause of growth inhibition.

5.1.3 Branches per plant

Branches per plant were significantly low at higher doses. Lower doses had a stimulatory effect on branches per plant which were higher than control. Mohideen (1988) in amaranth, Dharan (1993) in winged bean, Animon (1996) in bhindi, Nair (1996) in limabean and John (1997) in bhindi observed similar trend. The negative shift in higher doses has been described as a gradual phenomenon attributed to the occurrence of deleterious or harmful mutation whose frequency was more than the mutations of desirable nature (Virupakshappa *et al.*, 1980).

5.1.4 Leaf length and leaf width

Leaf length was significantly higher in lower doses than the control and showed an inverse relationship with increase in dose in both the species. Gamma rays had similar effect on leaf width also. Similar results have been reported by Mohideen (1988) in amaranth and John (1997) in okra.

5.1.5 Leaf stem ratio

A decreasing trend was observed for leaf stem ratio in both the species with increase in doses of gamma rays. It can be attributed to more leaf area in lower doses compared to the rest of the treatments.

5.1.6 Days to 50 percent bolting

The effect of gamma irradiation on flowering revealed a dose dependent relationship indicating a relationship between the dose administered and days taken to flowering. Number of days taken to flowering increased with increase in dosage of gamma rays. Higher doses of gamma rays may be lowering the rate of metabolic activities in the plants in their vegetative stage and thus leading to a prolonged vegetative phase and late flowering and the reverse may be the case at lower doses. The conclusions obtained by Dharan (1993) in winged bean, Veeresh *et al.* (1995), Animon (1996) in bhindi, Nair (1996) in limabean, John (1997) in bhindi and Munishamanna *et al.* (1998) in limabean indicate the same.

5.1.7 Vegetable yield

A gradual reduction of yield with increase in doses of gamma rays was noticed. Vegetable yield per plant was higher than control in lower doses of 50, 150 and 200 Gy in the case of *A. tricolor* (Arun) while in *A. hypochondriacus* all doses ranging from 50 Gy to 300 Gy had more vegetable yield per plant and were on par with control while lesser yield was noted for 350 Gy and 400 Gy (152 g/plant). At higher doses, there was reduction in vegetable yield.

The yield in any crop is a complex character determined by several components. Plant height, number of branches, leaf length and width are some of the contributing characters in lower doses were higher which in turn may have resulted in higher vegetable yield. Growth rate reduction at higher doses was attributed to abnormal cytological behaviour due to chromosomal damage and meiotic inhibition (Sparrow *et al.*, 1952), Dharan (1993) in winged bean, Gunasekaran *et al.* (1996) and Mohanasundaram *et al.* (1997) in cowpea obtained similar results.

5.1.8 Crop duration

The mean values for crop duration showed an increasing trend with increasing dosages of gamma rays. Similar reports have been obtained by Krishna (1985) and Animon (1996) in bhindi. Prolonged crop duration at higher doses can be attributed to delayed flowering which in turn prolonged the duration of vegetative phase.

5.1.9 Morphological variations and chlorophyll chimeras

Morphological abnormalities like spreading plants, variations in leaf shape and size etc. were observed in the M_1 generation. Mutagenic treatment may lead to abnormalities in stems, leaves, buds, branches, flowers and fruits. Morphological variants have been reported in M_1 generation by Oommen and Gopimany (1984) and Mini (1989) in cowpea after gamma irradiation. These morphological variations may be attributed to chromosome breakage, disrupted auxin synthesis and transport, disruption of mineral metabolism and accumulation of free aminoacids, as cited by Gunckel and Sparrow (1961).

5.1.10 Leaf spot disease

There was no significant difference between the treatment for disease severity and disease incidence in M_1 generation which indicate that irradiation did not have any effect on leaf spot disease in amaranth in M_1 generation. A similar study conducted by Animon (1996) in bhindi revealed no change in pests and disease incidence in the M_1 generation by irradiation treatment. Leaf spot disease scoring was taken at 15^{th} day after transplanting. Severity was very low ranging form 10-15 per cent in the initial growth period of the crop (15 DAT). Afterwards there was no disease at 30 DAT and 45 DAT. A rainfall of 52 mm and 91 per cent relative humidity are the contributing factors, which influenced the occurrence of disease in the early growth period. In later stages, absence of rainfall and low humidity (69%) resulted in the absence of the disease.

5.1. 11 Biochemical attributes

The range of values recorded for vitamin C, and Beta carotene content in amaranth in this experiment were in line with those reported by Mathew (2000). Higher content of Beta carotene in both the species and vitamin C in *A. hypochondriacus* in the lower doses were recorded. High carotene content in red pepper mutants (Batikyan *et al.*, 1981) and improved vitamin C content by mutation in *Momordica charantia* (Rajasekharan and Shanmugavelu, 1984) have also been reported.

The oxalate content ranged from 0.96 to 3.23 per cent and the nitrate content from 1.08 to 3.53 per cent in this study in M_1 generation. The range of values reported in this study is more or less same as that reported by Marderosian *et al.* (1980), Mallika (1987) and Krishnakuamry (2000). Oxalate content and nitrate content were seen increasing with increasing dose. At lower doses, content may be low because of the dilution of the antinutritional factors in plant tissues by increased growth.

5.2 INDUCED MUTATIONS IN M_2 GENERATION

From practical point of view the phenomenon of mutations can be divided into macro and micromutations (Gaul, 1964). Macromutations can easily be recognized on a single plant and can easily be selected in the M_2, but micromutations can only be detected in a group of plants by statistical methods.

5.2.1 Micromutations

The induction of polygenically controlled metric traits can be detected by the estimation of mean and variance of progenies in normal looking mutagen treated population.

5.2.1.1 Effects on morphological character and yield

In M_2 generation, number of branches per plant, leaf length, plant height at maturity, plant height at flowering, leaf stem ratio and vegetable yield per plant showed positive change at lower doses and negative change at higher doses in both the Amaranth species. There was no significant variation among the treatments and between the two Amaranth species for leaf width. Leaf length was recorded highest at 50 Gy dose. Number of branches were reduced at higher doses. In general, a progressive decline with increased dosages of gamma rays were observed for the above characters. Similar results were obtained by Mohideen (1988) in amaranth, and John (1997) in bhindi. A positive shift in mean value at lower doses was prominent in the case of plant height. It is the magnitude of the phenotypic effect of a mutation which gives the positive shift in mean value. A reduction in mean values for leaf stem ratio and vegetable yield per plant was noticed with increasing dosages of gamma rays. Yield is a complex character determined by several components. The yield contributing character like plant height, branches per plant, leaf length etc. are responsible for the positive shift in mean values for yield at lower doses and the reverse for higher doses.

LekhaRani (1985) reported a reduction in mean value for quantitative characters with increasing doses of mutagens in chilli. Similar opinion was given by Dharan (1993) in winged bean. Shift in mean values for growth and yield characters indicate the micromutations and emphasise the possibility of micromutational studies in further generations. Deepa (1995) in her investigations on winged bean concluded that the reduction in growth and yield character at higher doses of mutagen treated material was attributed to abnormal cytological behaviour due to chromosomal damage and meiotic inhibition. Gunasekaran *et al.* (1996) also reported a shift in mean values towards negative direction in the case of plant height and yield in cowpea with increase in doses of gamma rays. In *A. hypochondriacus*, lower doses of 100 Gy showed early flowering and in rest of the treatments flowering was late. A general conclusion drawn from earlier studies clearly demonstrates that mutation can create genetic variation in any direction. Earlier flowering at lower doses might be due to higher metabolic activities in the vegetative phase. These results are in corroboration with the findings of Deepa (1995) in winged bean, Nair (1996) and Mohanasundaram *et al.* (1997) in cowpea. Duration of the crop varied significantly among the treatment and within the species. In *A. tricolor* (Arun), all treatments were on par with control. The duration was shortest for 100 Gy and maximum for control in *A. hypochondriacus*. John (1997) in her evaluation in bhindi observed similar results of prolonged crop duration at higher doses. This can be attributed to delayed flowering which in turn prolonged the total duration of vegetative phase.

5.2.1.2 Leaf spot disease

Disease incidence was recorded 100 per cent in *A. tricolor* (Arun). *A. hypochondriacus* is immune to leaf spot infection.

Significant difference was observed between the treatments for disease severity. *A. tricolor* (Arun) is a highly susceptible cultivar to leaf spot disease recording 50.4 per cent disease severity in control at 30^{th} day after transplanting. In the present investigation, plants belonging to 100 Gy dose showed less disease severity (24.8%) compared to the rest of the treatments. Control and 400 Gy showed maximum disease infection (Fig.7) and based on disease severity, they were classified into highly susceptible group where as plants belonging to 100 Gy dose were classified as moderately resistant group. Remaining treatments belonged to moderately susceptible group. Gamma rays and EMS both have been successfully used to induce mutations for disease resistance in crop plants as has been reported by Sharma *et al.* (1989) in wheat, Motagi *et al.* (2000) in groundnut and Singh and Singh (2000) in bhindi. Disease severity was maximum at 30^{th} day after transplanting in all the doses as well as control. Observations on meteorological data revealed the occurrence of high rainfall (112.15 mm) and high relative humidity (94%) during this period. High rainfall and humidity are the factors favourable for the development and spread of leaf spot disease in amaranth. In the present investigation, induction of mutations for leaf spot resistance in amaranth was taken up as a preliminary trial and plants showing moderate resistance were identified. With further evaluation in the next generations, it may be possible to identify plants showing resistance to leaf spot infection and whether this character is stable in the next generation.

5.2.1.3 Quality attributes

Quality of amaranth is determined by the content of nutritional factors like vitamins, Beta carotene and the contents of antinutritional factors like oxalate and nitrate. In the present investigation, an improvement in vitamin C content in

A. tricolor (Arun) and Beta carotene content in *A. hypochondriacus* were analysed at lower doses. The average content of Vitamin C in *A. tricolor* (Arun) and Beta carotene was in accordance with those analysed by Mathew (2000). The range of values obtained for oxalate content and nitrate content were in conformity with those obtained by Krishnakumary (2000). Lower doses (50 Gy and 100 Gy) showed less nitrate content in *A. hypochondriacus* and it was on par with control in *A. tricolor* (Arun). This suggests that chemical content of the mutants vary with mutation dose. Similar observations of difference in phytic acid content in *Phaseolus vulgaris* and BOAA content in *Lathyrus sativus* were reported by Lolas and Markakis (1975) and Gottschalk and Wolff (1983) respectively.

5.2.2 Macro mutations and viable mutations

The spectrum and frequency of viable mutations was wider in *A. hypochondriacus* than in *A. tricolor* (Arun). In both the species, it was seen that 100 Gy and 250 Gy doses were most effective in inducing macromutations. In *A. tricolor* (Arun), mutation frequency was higher in 250 Gy (5.02) than 100 Gy dose (2.56). The difference in the frequency of viable micromutations in the two species can be due to varietal sensitivity. Similar reports have been obtained by Singh *et al.* (2000) in okra where gamma rays ranging from 15-60 kR were effective to induce viable macromutations. The macromutations affected the expression of normal character of plant and based on desired characters, 8 economic mutants were identified in the present experiment. High yielding and branching mutants were obtained in *A. tricolor* (Arun) while dwarf and early flowering mutants were obtained in *A. hypochondriacus*. Behera and Patnaik (1979) and Mohideen (1988) obtained a number of macromutations in *Amaranth spp.*

In present investigation, it became clear that the dose range tried for gamma rays is capable of causing variability in seed germination, survival, growth, flowering, yield contributory factors, leaf spot infection, chlorophyll variations and morphological abnormalities. Stimulatory effect was noticed at lower doses of gamma rays which may be due to the reason that, under certain conditions, the mutagens may

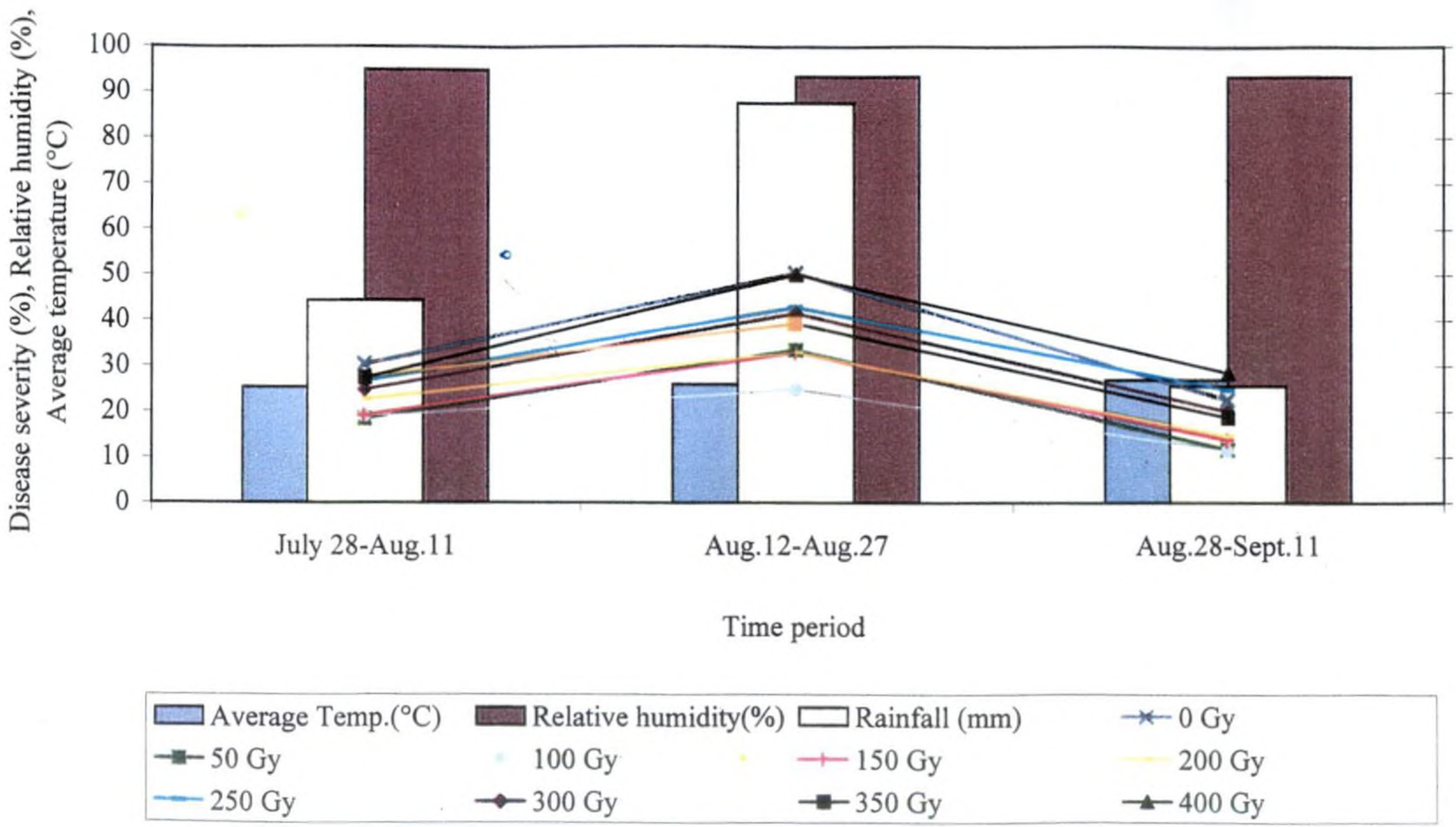


Fig. 7. Weather parameters and leaf spot infection during July 2002 to September 2002 in the M_2 generation

A. tricolor (Arun) and Beta carotene content in *A. hypochondriacus* were analysed at lower doses. The average content of Vitamin C in *A. tricolor* (Arun) and Beta carotene was in accordance with those analysed by Mathew (2000). The range of values obtained for oxalate content and nitrate content were in conformity with those obtained by Krishnakumary (2000). Lower doses (50 Gy and 100 Gy) showed less nitrate content in *A. hypochondriacus* and it was on par with control in *A. tricolor* (Arun). This suggests that chemical content of the mutants vary with mutation dose. Similar observations of difference in phytic acid content in *Phaseolus vulgaris* and BOAA content in *Lathyrus sativus* were reported by Lolas and Markakis (1975) and Gottschalk and Wolff (1983) respectively.

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In present investigation, it became clear that the dose range tried for gamma rays is capable of causing variability in seed germination, survival, growth, flowering, yield contributory factors, leaf spot infection, chlorophyll variations and morphological abnormalities. Stimulatory effect was noticed at lower doses of gamma rays which may be due to the reason that, under certain conditions, the mutagens may even result in an increase in the physiologically active growth promoting substances, perhaps by the destruction of inhibitory substances (Casarett, 1968).

The selected progenies in M_2 should be advanced to subsequent generations and the practical utility of these selected mutants have to be fully assessed by evaluating them in M_3, M_4 and further generations.

Summary

6. SUMMARY

The present investigation on 'Induction of leaf spot resistance and improvement in quality of amaranth through mutation' was carried out in the vegetable research field of the Department of Olericulture, College of Horticulture, KAU, Vellanikkara. The objective of the study was to standardize the optimum dose of the physical mutagen (gamma ray) for inducing variability and also to study the differential response of the mutagen on growth parameters, leaf spot infection and biochemical attributes in M_1 and M_2 generations in two amaranth species viz. *Amaranthus tricolor* (Arun) and *A. hypochondriacus* (A-204).

For estimating the LD_{50}, seeds of the two species were treated with 7 doses of gamma rays from 100 Gy to 1000 Gy and sown in pots along with control. The results of this study revealed that there was a progressive decline in germination and survival percentage with increase in doses of gamma rays in both laboratory and field conditions with maximum germination and survival percentage observed for control. Since doses above 400 Gy showed reduction in survival percentage lesser than 50, 400 Gy was fixed as the LD_{50} and doses below 400 Gy (50, 100, 150, 200, 250, 300, 350 and 400 Gy) were tried to assess the performance of the plants in the field.

In the M_1 generation, there was reduction in plant height with increased in doses of gamma rays. Lower doses ranging from 50 Gy to 200 Gy showed more mean leaf length and leaf width which were higher than control. A decreasing trend could be noticed for number of branches per plant and leaf stem ratio with increase in dosages of gamma rays. Vegetable yield was maximum at 50 Gy (305 g/plant) which was much higher than control in *A. tricolor* while in *A. hypochondriacus*, more vegetable yield was noticed for all treatments upto 300 Gy. Days to 50 per cent bolting and crop duration was found to be early in lower doses of gamma rays.

Leaf spot disease was noticed only in *A. tricolor* (Arun) and it was not significantly different between the treatments in M_1 generation with a disease severity

of 10-15 per cent. *A. hypochondriacus* was immune to leaf spot infection. Highest content of Beta carotene was found at 50 Gy dose in both *A. tricolor* (7561 I.U) and *A. hypochondriacus* (3442 I.U). Highest content of vitamin C was recorded at 100 Gy in *A. hypochondriacus* while untreated plants had more vitamin C content in *A. tricolor*. Mean oxalate content and nitrate content was very much lower at lower dose of 50 Gy than control. The content of both the antinutritional factors (oxalate and nitrate) increased with increase in doses.

The seeds from the M_1 generation were carried forward to the M_2 generation to assess the induced variability for the various growth characters and other attributes. In *A. tricolor*, plants were tallest at 50 Gy dose (78 cm) while maximum plant height was noticed at 250 Gy (134 cm) in *A. hypochondriacus*. Mean leaf length was found to be more at lower doses of 50 Gy and 100 Gy in both species. Mean number of branches per plant and mean leaf stem ratio were lower in all the doses above 250 Gy than control. Days to bolting and crop duration was earlier than control at 100 Gy dose in *A. hypochondriacus* while significant differences were not noticed between the treatments for these characters in *A. tricolor*. Vegetable yield per plant was maximum at 50 Gy dose in *A. tricolor* while it was maximum at a dose of 250 Gy in *A. hypochondriacus*.

In *A. tricolor*, 100 per cent leaf spot disease incidence was noticed in all the treatments. Disease was found to be maximum for control and 400 Gy with a severity of around 50 per cent whereas it was minimum for 100 Gy (25%). Hence, based on disease severity score, 100 Gy dose was classified as moderately resistant control and 400 Gy as highly susceptible and rest of the treatments as moderately susceptible.

Beta carotene content was found to be higher and comparable with control in lower doses of 50, 100 and 150 Gy in *A. tricolor* while in *A. hypochondriacus* the content was significantly higher than control at 100 and 150 Gy with a value of 3758 I.U. in 150 Gy. Similarly vitamin C content of both the species were higher at lower doses and in *A. tricolor*, it was higher than control. The antinutritional factors like oxalate and nitrate were maximum at higher doses.

The spectrum of viable mutations was wider in *A. hypochondriacus* than *A. tricolor* in M_2 generation. In *A. hypochondriacus* it was 9.2 at 100 Gy dose followed by 250 Gy (4.66) and 350 Gy (1.99). More branching, dwarf plants, early flowering, fasciation, reduced leaf size, presence of leaflets and inflorescence mutant were observed randomly. In *A. tricolor*, mutation frequency was higher at 250 Gy (5.02) followed by 100 Gy (2.56).

From the present experiment, the results revealed that at a dose of 100 Gy the plants had less leaf spot severity when compared to control. Further research on the same is required to confirm it. Based on the desirable characters, eight economic mutants viz., high yielding mutants, branching mutants, dwarf mutants, early flowering mutants and inflorescence mutants were identified. Detailed analysis on the extent of variability created by gamma rays and the selection of the desirable types in M_3, M_4 and further generations are suggested as the future line of work in this particular crop.

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*Originals not seen

Appendices

Appendix I

Analysis of variance for morphological and biochemical characters due to gamma irradiation in M_1 generation.

Characters		Source		
		Genotype (df=1)	Dose (df=8)	Genotype x Dose Interaction (df=8)
Germination	MSS F value	50.074 13.7959	545.50 30.5017**	21.491 1.2017
Survival	MSS F value	32.667 0.8155	462.583 30.8104**	17.25 1.1489
Plant height of harvest	MSS F value	6152.536 78.9055**	79.893 7.5465**	25.396 2.3989**
Plant height at flowering	MSS F value	10475.917 83.6705**	143.79 10.6022**	31.255 2.3045*
Branches per plant	MSS F value	429.542 45.5283*	6.972 11.5662**	0.931 1.544
Leaf length	MSS F value	31.526 2.3139	11.42 20.2909**	0.723 1.2841
Leaf width	MSS F value	165.795 143475.8418**	1.912 14.2436**	0.122 0.9123
Days to 50 per cent bolting	MSS F value	1472.667 238.8108**	7.769 3.4598**	7.333 3.266**
Vegetable yield per plant	MSS F value	112066.67 24.5470*	2411.06 9.8084**	744.208 3.0275**
Leaf stem ratio	MSS F value	0.266 147.8905**	0.071 3.4948**	0.034 1.6627
Crop duration	MSS F value	1296605.910 0.6438	6769508.363 11.1768**	2782185.158 4.5935**
Vitamin C	MSS F value	27398.286 13.0568	7073.811 52.7307**	1329.403 9.9098**
Oxalate	MSS F value	2.518 268.262**	2.009 21.3298**	0.165 1.7539
Nitrate	MSS F value	28.297 367.7299**	0.763 33.8683**	0.126 5.589**

* Significant at 5% level

** Significant at 1% level

Appendix II

Analysis of variance for morphological and biochemical characters due to gamma irradiation in M_2 generation.

Characters		Source		
		Genotype (df=1)	Dose (df=8)	Genotype x Dose Interaction (df=8)
Germination	MSS	352.667	207.083	17.583
	F value	54.2564*	11.8994**	1.0104
Survival	MSS	60.167	344.50	14.333
	F value	2.9835	20.6614**	0.8596
Plant height of harvest	MSS	20592.041	251.153	155.748
	F value	480.3304**	3.3912**	2.103
Plant height at flowering	MSS	23575.202	293.786	261.91
	F value	197.4629**	3.302**	2.9437**
Branches per plant	MSS	338.501	8.221	3.307
	F value	345.8828**	4.4764**	1.8004
Leaf length	MSS	30.933	3.502	1.723
	F value	8.1127	2.4733*	1.2166
Leaf width	MSS	82.337	0.725	0.208
	F value	149.3405**	1.4662	0.4205
Days to 50 per cent bolting	MSS	244.907	356.50	397.241
	F value	51.0618*	33.2631**	37.0644**
Vegetable yield per plant	MSS	50367.178	2485.339	1797.65
	F value	53.7161*	5.6292**	4.0716**
Leaf stem ratio	MSS	2.353	0.056	0.035
	F value	4.7750	2.5633*	1.5792
Crop duration	MSS	0.074	299.75	317.7823
	F value	0.0816	31.8946**	33.7823**
Beta carotene	MSS	12576652.803	435412.993	373863.872
	F value	7.7206	3.555**	3.0524**
Vitamin C	MSS	211.274	1868.22	749.858
	F value	0.1639	6.7098**	2.6931*
Oxalate	MSS	0.053	1.345	0.728
	F value	0.0416	5.8513**	3.1656**
Nitrate	MSS	1.135	3.754	0.544
	F value	3.26	59.7410*	8.6636*

* Significant at 5% level

** Significant at 1% level

Appendix III

Weather parameters during first crop period

	Temperature (°C)		Humidity (%)	Rainfall (mm)
	Maximum	Min.		
October 2001				
1st week	30.1	23.1	93	47.6
2nd week	30.1	23.0	91	94.0
3rd week	31.0	22.8	90	56.2
4th week	31.5	22.3	91	61.5
5th week	31.8	23.5	90	11.3
November 2001				
1st week	31.2	23.6	92	30.6
2nd week	31.4	23.2	91	74.3
3rd week	31.7	23.3	73	0.0
4th week	31.2	22.1	72	0.0
December 2001				
1st week	31.5	22.3	72	0
2nd week	31.1	18.9	67	0
3rd week	30.9	23.2	74	0
4th week	32.1	23.8	76	0
January 2002				
1st week	32.4	23.7	70	0
2nd week	32.5	23.0	81	0
3rd week	32.5	21.3	76	0
4th week	33.4	23.4	88	0
5th week	31.1	23.4	69	0

Appendix IV

Weather parameters during second crop period

	Temperature (°C)		Humidity (%)	Rainfall (mm)
	Maximum	Min.		
July 2002				
1st week	30.3	23.6	94	57
2nd week	29.4	23.1	94	126
3rd week	29.7	22.7	95	58
4th week	29.9	22.9	93	70.4
5th week	28.1	22.5	95	83.6
August 2002				
1st week	28.6	22.2	95	94
2nd week	27.9	22.8	94	337
3rd week	30.1	23.4	93	13.8
4th week	30.9	24.1	93	3.8
September 2002				
1st week	29.8	23.2	94	98.7
2nd week	30.7	22.9	92	-
3rd week	31.3	22.8	91	-
4th week	32.5	22.7	90	21.5
October 2002				
1st week	32.2	23.3	89	51.0
2nd week	29.3	23.1	93	268.3
3rd week	30.1	23.0	92	25.1
4th week	31.5	23.5	92	9.9
5th week	31.6	23.3	84	33.4

INDUCTION OF LEAF SPOT RESISTANCE AND IMPROVEMENT IN QUALITY OF AMARANTH THROUGH MUTATION

By

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

The present study on 'Induction of leaf spot resistance and improvement in quality of amaranth through mutation' was carried out in the vegetable research field of the Department of Olericulture, College of Horticulture, KAU, Vellanikkara with the objective of standardizing the optimum dose of gamma ray for inducing variability and to study the differential response of the mutagen on growth parameters, leaf spot infection and biochemical attributes in M_1 and M_2 generation.

Based on the survival percentage the LD_{50} was fixed as 400 Gy and doses below that were tried. In general, a progressive decline in germination percentage, survival percentage, plant height, branches per plant, leaf length, leaf width, leaf stem ratio and vegetable yield per plant was noticed with increase in dosages of gamma rays in M_1 generation. Increased content of Beta carotene, vitamin C and decreased content of oxalate and nitrate could be found at lower doses.

Analysis of induced variability in M_2 generation showed a negative shift in mean values for all the characters except days to flowering and crop duration. The mean performance was found to be minimum for higher doses and maximum for control and lower doses. Stimulatory effects could be noticed for plant height, leaf length, vegetable yield per plant, Beta carotene and vitamin C. This clearly demonstrated that a positive response to selection can be created by gamma rays in amaranth. Observations taken on disease severity at 30^{th} day after transplanting revealed that plants at 100 Gy dose showed less leaf spot disease infection whereas control and 400 Gy dose were highly susceptible to the disease.

Quantitative analysis of the viable mutations revealed that the spectrum was much wider in *A. hypochondriacus* than *A. tricolor*. With further research on the same, it may be possible to isolate desirable mutants in the further generations.