Influence of Continuous Gamma Irradiation on Morpho-agronomic Characteristics of *Amaranthus caudatus* in M1 and M2 Generations

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To cite this article:

Veer Singh Rawat, Shyam Shanker Singh, Mohd Rafi Wani, Ankita Singh. Influence of Continuous Gamma Irradiation on Morpho-agronomic Characteristics of *Amaranthus caudatus* in M1 and M2 Generations. *American Journal of Agriculture and Forestry*.

Vol. 5, No. 4, 2017, pp. 130-136. doi: 10.11648/j.ajaf.20170504.17

Received: May 28, 2017; **Accepted:** June 14, 2017; **Published:** July 24, 2017

Abstract: *Amaranthus caudatus* play an important role against hunger and malnutrition that occur due to low rain fall conditions, gaining a wide attention in food and medicinal industry. Being a versatile plant in terms of its nutritional value the improvement of this plant with reference to germination, growth and yield is still uncharacterized. Therefore the present investigation is carried out to determine the influence of gamma radiation in modifying germination, growth and yield attributes of *Amaranthus caudatus*. The air dried seeds were exposed to different gamma irradiation doses (10KR, 20KRC, 40KRC and 80KRC) using ⁶⁰Co source, sowing was carried out in a complete randomized block design, and the observations were taken upto M2 generation. The results showed that the influence of different doses of gamma irradiation on germination parameters was significant in M1 and M2 generations. The influence on the survival percentage was obtained in both M1 and M2 generation, and highest percentage of survival was recorded under 20KRC in both M1 and M2 generations. The average yield per plant in M1 and M2 generations were observed higher under 20KRC dose level of gamma irradiations. The gamma irradiation treatment showed significant enhancement in different growth parameters in both M1 and M2 generations. Also, it was observed that the growth and yield in M2 generation across all doses was better than M1 generation.

Keywords: *Amaranthus caudatus*, Gamma Irradiations, Growth, Yield, M1 and M2 Generation, Morpho-agronomic Characteristics

1. Introduction

Amaranthus caudatus (family Amaranthaceae) is underutilized pseudo cereal that can play an important role against hunger and malnutrition that occur due to low rainfall conditions. It is a broad-leaved non-grass plants that produce significant amounts of edible cereal like grains having an exceptional nutritive value. Amaranth is very versatile as a food ingredient and can diversify farming enterprise, as it can be expected to prevent food deficit and to feed the world [1]. *Amaranthus caudatus* grows rapidly and has a high tolerance to arid conditions and poor soils where traditional cereals cannot be grown. *Amaranthus caudatus* seeds with their phenomenal nutritional profile provide several important

nutrients that are often difficult to incorporate into a restrictive diet. The seeds contain large amounts of dietary fiber, iron, and calcium. It also have high amounts of lysine, methionine and cysteine, combined with a fine balance of amino acids, making them an excellent source of high quality, balanced protein, which is more complete than the protein found in most grains. In addition to its outstanding nutritional value, amaranth is also very low in sodium and contains no saturated fat [2]. The increasing world population has raised concerns over food security. In order to feed the world, the projected target is to double food production by 2050. However, to fulfill these objective plants with better growth and yield should be preferred. So, from last few years new technologies have been developed to improve the

growth and yield of agriculture crops. Across all the techniques, gamma rays induced crop improvement is one which has shown a possible gain in the germination, morpho-agronomic traits of different agriculture crops within less time.

Gamma rays can cause multiple alterations in plant both morphologically and physiologically through direct and indirect interactions with an organism [3-4]. Gamma rays produce free radicals, which can damage various important compounds in plant cells and brings changes in morphology, growth, biochemical and physiological level [5-6]. Some of these include visible changes such as abnormal shape or appearance, reduced growth and reduced reproductive capacity [7-8], and physiological alterations such as accumulation of anthocyanin, expansion of root radial cells, induction of trichome formation, dilation of thylakoid membranes, change of photosynthesis, modulation of antioxidative system [9-13]. Several positive mutations have been created in agricultural crops by using gamma irradiations. Crops with improved characteristics have successfully been developed by mutagenic inductions [14-15]. Considering the effects of radiation on plants, the present study was conducted to determine the effects of gamma irradiation on *Amaranthus* germination and some Morpho-agronomic characteristics and its yield in M1 and M2 generations.

2. Material and Methods

The air dried seeds were irradiated with different doses of gamma rays (10KR, 20KR, 40KR and 60KR) using a ^{60}Co gamma source with strength of 100 rad/sec. The dose regime selection was based on the previous studies treatment on genus *Albizia*, *Bauhinia* and *Robinia* [16-17] where significant results on different germination and growth attributes were recorded under different gamma irradiation treatments.

Hundred seeds were treated for each dose. Each treatment was replicated four times with 25 seeds in each replicate and the whole experiment was arranged in a Randomized Block Design. Seeds without treatment were taken as control samples and the results of irradiated seeds were compared with these untreated seeds. The experimentation for germination early growth behaviour has been carried out in petridishes. Seeds were washed with distilled water and then shifted to petridishes. Whatmann filter paper No. 1 have been used in petridishes. For studying the detailed growth behaviour the seeds have been grown in the first week of May in field having well drained and pulverized soil and fairly leveled plot. In order to observe the transmission of the modifications in different morpho-agronomic characteristics induced by different gamma irradiation doses in *Amaranthus caudatus*, the experiment was carried upto M2 generation for which the seeds of M1 generation for each treatment were collected and sown in the field following the methodology as designed for M1 generation

Emergence of radicle upto 2mm long has been treated as

germinated seeds [18]. The germinated seeds were counted daily from the starting of the experiment upto the end of the experiment and then the germination percentage was calculated.

Germination energy index (GEI) was calculated from daily germination record. For germination energy index an appropriate record of newly germinated seeds were made and GEI was calculated by equation..(1)

$$GEI = \frac{A_1 + (A_1 + A_2) + (A_1 + A_2 + A_3) + (A_1 + A_2 + A_3 + \dots + A_n) \times 100}{Y \times N} \quad (1)$$

Where $A_1, A_2, A_3, \dots, A_n$ is the number of seeds newly germinated on 1,2,3... and n^{th} days, respectively. N is the total number seeds used for the treatment, and Y represents the number of days for each observation.

For germination value (GV) germination data were considered upto the day when germination became constant for three consecutive days (peak value). The same day was used as a reference point for computing the germination value and was calculated by equation (2) [19].

$$G.V. = MDG \times PV \quad (2)$$

Where MDG is mean daily germination and PV is the peak value.

Analysis of shoot length, root length, leaf length, average leaf area and average yield per plant were measured on the final day of experiment. Plants under different treatments of gamma rays and control set have been uprooted after 92 days of planting in M1 generation. For M2 generation, plants under different treatments have been uprooted after 105 days of planting. The relative growth rates of the plants were determined in terms of their fresh and dry weight. Dry weight was calculated by keeping the seedlings in an oven at 80°C for 24hours.

The data was subjected to analyses of variance (ANOVA) in a randomized block design. Treatment means were compared using the Duncan Multiple Range Test [20] at ($P \leq 0.05$) level of significance.

3. Result

The effect of different gamma irradiation doses on germination in M1 and M2 generation is given in Figure 1 to Figure 4. No more significant difference in germination percentage was recorded in treated groups in both M1 and M2 generation. However a significant variation in germination energy index was observed in treated experimental set of both M1 and M2 generation than the control. The maximum germination energy index (100 ± 1.24) was observed under 60KRC in M1 generation than the control (95 ± 2.22). However the overall germination energy index in M2 generation under all tested doses were better than M1 generation. The lower gamma irradiation upto 20KRC didn't show a significant variation in germination value than the control, in contrast the doses above 20KRC significantly reduced ($P \leq 0.05$) the germination value than the control.

Figure 1. *Amaranthus caudatus*- Influence of continuous doses of gamma rays on germination percentage and germination energy index value (M1 generation).

Figure 2. *Amaranthus caudatus*- Influence of continuous doses of gamma rays on germination percentage and germination energy index value (M2 generation).

Figure 3. *Amaranthus caudatus*- Influence of continuous doses of gamma rays on germination value (M1 generation).

Figure 4. *Amaranthus caudatus*- Influence of continuous doses of gamma rays on germination value (M2 generation).

Figure 5. *Amaranthus caudatus*- - Fresh weight and dry weight of seedling as influenced by different continuous doses of gamma rays after 7 days (M1 generation).

Figure 6. *Amaranthus caudatus*- - Fresh weight and dry weight of seedling as influenced by different continuous doses of gamma rays after 7 days (M2 generation).

Fresh weight of seedling in M1 and M2 generations were stimulated by different gamma irradiation doses (Figure 5 and Figure 6). The maximum enhancement of seedling fresh weight in M1 generation was recorded under the treatment of 10KRC (Figure 5) than the control, and in M2 generation the highest fresh weight was recorded for the seedlings treated with 20KRC (Figure 6) than the control. In contrast the dry weight of the seedling didn't show any significant difference across all the treated seedlings in both M1 and M2 generation.

Figure 7 and Figure 8 reveals that the survival percentage significantly increased ($P \leq 0.05$) by gamma irradiation exposure in both M1 and M2 generations. In M1 generation the higher doses were found to be lethal and increased the mortality rate. However the lower doses stimulated the survival percentage in M1 generation and gaining its maximum value under 20KRC treatment. The highest mortality was recorded under 60KRC dose treatment. In contrast, the survival percentage in M2 were found higher under all treated doses than untreated plants. The 20KRC

treatment showed maximum survival percentage, followed by 10KRC treatment (Figure 7 & 8).

Figure 7. *Amaranthus caudatus*-Survival percentage as influenced continuous doses of gamma rays (M1 generation).

Figure 8. *Amaranthus caudatus*-Survival percentage by different as influenced by different continuous doses of gamma rays (M2 generation).

The average grain yield per plant (Figure 9&10) has significantly increased ($P \leq 0.05$) under 20KRC treatment, followed by 40KRC in comparison to control set. However the other doses don't show any significant difference than the control in M1 and M2 generation. The grain yield increased upto 66% in M2 generation in comparison to the control.

Figure 9. *Amaranthus caudatus* - Average yield per dose per plant as influenced by different continuous doses of gamma rays (M1 generation).

Figure 10. *Amaranthus caudatus* - Average yield per dose per plant as influenced by different continuous doses of gamma rays (M2 generation).

Table 1. Average root length, shoot length, number of leaves, leaf length and number of branches per plant in *Amaranthus caudatus* under influence of different doses of gamma radiations (M1 generation) after 92days of sowing.

Dose	Average Root Length/plant (cm)	Average Shoot Length/plant (cm)	Average No. of Leaves/plant	Average Leaf Area /plant (cm ²)	Average Leaf length /plant (cm ²)	Average number of branches
Control	4.31±0.21 ^a	20.5±1.02 ^{ab}	12.4±0.44 ^c	1975.1±3.56 ^b	11.3±0.56 ^a	5.31±0.37 ^b
10KRC	5.08±0.46 ^a	19.0±2.10 ^b	12.7±1.21 ^c	1935.2±5.68 ^b	10.8±0.94 ^a	6.98±0.52 ^a
20KRC	5.18±0.12 ^a	18.3±0.94 ^b	17.6±1.66 ^a	2275.2±4.21 ^a	11.2±1.32 ^a	6.66±0.94 ^a
40KRC	4.96±0.35 ^{ab}	19.1±1.74 ^b	14.3±0.87 ^b	2235.3±6.67 ^a	11.2±0.17 ^a	5.01±0.86 ^b
60KRC	5.64±0.17 ^a	22.2±1.09 ^a	10.1±0.22 ^d	1636.5±3.54 ^c	11.4±1.10 ^a	4.28±0.72 ^c
ANOVA	*	*	*	**	NS	*
One Way						

Means within a column followed by same letter are not significantly different ($P \leq 0.05$). The data shown are mean $\pm$ SE of four replicates. Different letters a, b, c and d denote significant difference ($p \leq 0.05$) between the treatments.

*Statistically significant difference at $P \leq 0.05$

** Statistically significant difference at $P \leq 0.001$

The results doesn't show any significant increase in the average shoot and root length per plant in M1 generation,

however a slight enhancement in the shoot length was recorded by the seedlings subjected to 60KRC gamma

irradiation dose but it was not significant (Table 1). The average number of leaves and leaf area per plant (Table 1) was stimulated significantly by 20KRC (17.6 ± 1.66 , 2275.2 ± 4.21) in comparison to the control (12.4 ± 0.44 , 1975.1 ± 3.56). The 60KRC dose inhibited the leaf formation and reduced the average leaf area significantly ($P \leq 0.05$) than the control set. The highest number of branches per plant were formed under 10 KRC treatment (6.98 ± 0.52), followed by 20KRC (6.66 ± 0.94) than the control (5.31 ± 0.37).

Different growth parameters showed a significant difference under different treatments, when the plants were observed upto M2 generation (Table 2). The results confirm that the gamma irradiations can be better tool in modifying the different growth parameters upto number of generations. In M2 generation a significant enhancement in average root

and shoot length have been observed under the 40KRC (8.49 ± 0.35 , 28.03 ± 2.17) than under the control (4.31 ± 0.21 , 13.44 ± 1.12). An overall two fold enhancement of these parameters was recorded in M2 generation. Average leaf length and leaf area also enhanced significantly across all treated seedlings. The highest numbers of leaves were formed in the plants treated at 40KRC and 60KRC dose level (Table 2), whereas the other treatment doesn't record any significant variation than the control. The average leaf area increased significantly in M2 generation, gaining the maximum value in the seedling treated with 10KRC (5875.41 ± 11.42) than the control (0729.12 ± 06.58). Results obtained for the average number of branches per plant in M2 generation doesn't alter than the results obtained in M1 generation.

Table 2. Average root length, Shoot length, number of leaves, leaf length and number of branches per plant in *Amaranthus caudatus* under influence of different doses of gamma radiations (M2 generation) after 105 days of sowing.

Dose	Average Root Length/plant (cm)	Average Shoot Length/plant (cm)	Average No. of Leaves/plant	Average Leaf Area /plant (cm ²)	Average Leaf length /plant (cm)	Average number of branches
Control	4.31 ± 0.21^c	13.44 ± 1.12^c	09.20 ± 0.98^b	0729.12 ± 06.58^c	13.20 ± 0.64^c	4.20 ± 0.24^c
10KRC	5.67 ± 0.46^{bc}	12.40 ± 0.94^c	09.30 ± 0.42^b	5875.41 ± 11.42^a	17.30 ± 1.21^a	6.10 ± 0.46^a
20KRC	4.97 ± 0.12^c	11.14 ± 0.52^c	09.40 ± 1.02^b	2912.35 ± 09.32^c	12.20 ± 0.69^c	6.20 ± 0.21^a
40KRC	8.49 ± 0.35^a	28.03 ± 2.17^a	11.05 ± 0.69^c	3917.18 ± 06.64^b	13.40 ± 0.32^c	5.10 ± 0.30^{bc}
60KRC	6.62 ± 0.17^b	16.04 ± 1.66^b	11.60 ± 1.32^c	2775.12 ± 08.74^d	15.17 ± 0.94^b	5.00 ± 0.25^{bc}
ANOVA One Way	*	*	*	**	*	*

Means within a column followed by same letter are not significantly different ($P \leq 0.05$). The data shown are mean $\pm$ SE of four replicates. Different letters a, b, c and d denote significant difference ($p \leq 0.05$) between the treatments.

*Statistically significant difference at $P < 0.05$

** Statistically significant difference at $P < 0.001$

4. Discussion

With the help varied irradiation treatments productivity in *Amaranthus caudatus* can be increased. With rapid change in climate at global level, which influences productivity of crops, gamma rays can be handy for higher productivity [21] of plants. As the exposure of gamma irradiations is known to produce morphological, physiological and biochemical mutants. These improved mutants have the properties with high production of metabolites, and high productivity [22-24]. In the present study the the gamma irradiations were tested to modify the germination, growth and yield of *Amaranthus caudatus*. The results of the present study showed that gamma rays is an efficient tool for increasing growth and yield in *Amaranthus caudatus*. It was observed that there were significant effect on the plant development and production in the M1 and M2 generation.

The plant height variation under different irradiation treatments were observed in comparison to the control in M1 generation. This is in accordance with report [25-27] in soybean, observed that the exposure of gamma irradiation influence the plant height moderately. However in M2 generation it was observed that the plant height was significantly altered. The reports [28] in pigeon pea and in sorghum [29] showed enhancement effect in the plant height in M2 generation. They observed that plant height increased

with the application of gamma irradiation under the treatment of 40KRC and 60KRC. The stimulating effect of gamma irradiation on plant height may be due to stimulation of cell division or cell elongation, due to alteration of metabolic processes which affect synthesis of phytohormones and nucleic acids [30]. It was noted in present study that the gamma irradiations stimulate the root growth in both M1 and M2 generations. It was observed that gamma irradiations significantly increase the root growth in *Canscora decurrens* [31]. a stimulatory influence of gamma irradiations on root growth of *Terminalia tomentosa* [32]. The data in table 1 shows the maximum number of branches (6.98 ± 0.52) per plant were found under 10KRC and it was followed by 20 KRC (6.66 ± 0.94) than the control (5.31 ± 0.37). The minimum value for number of branches/plant was noticed under 60KRC treatment (4.28 ± 0.72). The stimulation in number of branches by lower doses of gamma irradiations and reduction in number of branches by higher doses of gamma irradiations in *Brassica napus* was noted [26]. The increased branches and leaves may be due to the enhanced production of IAA and kinetin which stimulates the production of large number of leaves and branches [33].

The results obtained in the present study (Table 2) indicates that there is a significant differences in the yield per plant in *Amaranthus caudatus* for M1 and M2 generations. The stimulation in the yield might be directly related to the

gene compliment, which might have been activated by the exposure of gamma treatments [34]. As, the similar results in the present study were seen in M2 generation thus the foresaid explanation (activation of genes) can be better confirmed. a significant increase of chickpea grain yield using gamma irradiation at 600 Gy was reported [29]. High yielding and early maturing barley plants were developed by mutation breeding methods [35]. Highest grain yield increase in soybean irradiated with 20KR of gamma rays was reported [27]. The increase in the grain yield can also be correlated with the increase in the average leaf area per plant by 20KRC and 40KRC treatments, which potentially increased the growth rate and hence stimulating the average grain yield of *Amaranthus caudatus*. The increase in the grain yield by the application of gamma irradiation treatments can be exploited for the commercial cultivation of treated *Amaranthus caudatus* crop to fulfill the gap existing in the food security problems of the country.

5. Conclusion

According to the obtained results, it was concluded that the gamma irradiation treatments significantly alter the different morpho-agronomic traits in *Amaranthus caudatus*. The effects of 20KRC and 40KRC irradiation treatments were recognized as radiopotential doses, as these treatments significantly increased the average grain yield per plant upto M2 populations. The selection of the treated plants with specific dose with a specific morpho-agronomic trait, can be preferred for the cultivation of *Amaranthus caudatus* at commercial base, in terms of the concerns over food security.

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