



Effect of X-Ray Irradiation on the Growth and Yield Parameters of Four Cowpea (*Vigna Unguiculata* (L.) Walp) Genotypes

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Abstract

Induced mutagenesis has been used as a tool in improving the agronomic traits of crops. The effects of different x-ray irradiation doses on vegetative and yield parameters of four cowpea (*Vigna unguiculata* (L.) Walp) genotypes (Black eye, Light brown eye, Dark brown eye and Ife brown) were evaluated. The seeds were subjected to various x-ray doses (0, 5, 10, 15 and 20 mGy). The experiment was set up in a 4×5 factorial experiment in randomized complete block design (RCBD) with three replicates. The result revealed that x-ray irradiation dose (10 mGy and 15 mGy) induced obvious variability in all the vegetative parameters (Days to 50 % emergence, plant height, number of leaves, leaf area, stem girth and number of branches). Yield parameters such as days to 50 % flowering, pod length, 100 seed weight, seed yield per pod (g) and grain yield per hectare (t/ha) were significantly increased to maximum by the different X-ray doses when compared to the control plants. Among the four varieties and treatments, light brown eye at 15 mGy performed better in yield when compared with the other varieties studied. Contrastingly, the high mutagenic dose of 20 mGy substantially decreased all the vegetative and parameters as compared to the control. Therefore, the overall result reveals that low x-ray irradiation doses of 10 mGy and 15 mGy could induce variability and enhance vegetative growth and yield of cowpea. Thus, it is recommended that x-ray be employed in the breeding program of cowpea.

1. Introduction

Mutation is the sudden change that occurs in the heritable trait inside the genetic makeup of a living organism not certainly by genetic segregation or combination instead induced by chemicals, physical or biological agents [1]. Mutagenesis in plants can be actualized by employing physical mutagens (ionizing and non-ionizing radiations) or chemical mutagens. Mutation breeding affords perfect and appealing choice for plant breeders to establish desired traits in plant [2]. In addition, it makes possible important attributed or traits that either cannot be found in nature or have been lost during evaluation [3]. Mutagenic treatment of plants alters genes or break chromosomes. Physical mutagens such as x-ray and gamma-ray cause physicochemical effects therefore influencing the growth and physiological alteration in plants [4]. Many authors have reported the effect of ionizing radiation at higher doses as being harmful and hindering to plant growth, while other

have opined that lower ionizing radiation doses positively affects the growth of plant and productivity [5, 6, 7]. [8] reported that exposure of wheat plants to lower doses of radiation induce germination and growth improvements while higher doses result in growth aberrance, delay in germination or even death. Cowpea is commonly known in Nigeria as “beans”. It is a dicotyledonous plant of the Fabaceae family. It is a self-pollinating crop and its genetic diversity within varieties is relatively low [9]. Cowpea is a leguminous crop providing a sustainable source of protein for human and animal nutrition [10]. Cowpea is one of the most important staple food **crops** grown in Nigeria and in some regions of tropical Africa. It is an ancient food crop whose origin is said to be African, Nigeria and Ethiopia, Asia-Hindustan and even South America [11]. It plays a major significant role to the livelihoods of millions of poor people in less developed countries [12, 13]. Cowpea occupies a valuable position in the world agriculture due to its high protein composition, essential amino acids and its capability to fix atmospheric nitrogen and replenish soil nutrient [14]. According to [15], Nigeria is the world’s producer of cowpea with an annual production of 2,950,000MT in 2013. Nevertheless, in Nigeria cowpea productivity and production is hindered by many setbacks such as rainfall (in the southern part, which makes the crop to be seasonal) pest, and diseases per cultural practices, drought, low fertility, crop protection practices, high temperatures, a narrow genetic base for breeding as well as low pH.

[16] have stated that induced mutation will advance to possess an increasing role in developing crop varieties with desirable traits as a major component of environmentally sustainable agriculture. Induce mutation and its breeding principles are vital tool for improving both quantitative and qualitative traits in crop plants within the possible short time frame than conventional breeding. Although the response of cowpea to gamma rays and other chemical mutagens have been reported however, there is not much information on the physiological response induced by x-ray irradiation on cowpea. Therefore, this study was undertaken to ascertain the x-ray irradiation effect on the vegetation parameters of four genotypes of cowpea.

2. Methodology

Four cowpea genotypes identified as (Ife brown, white black eye, white dark brown eye, and white light brown eye) where locally sourced within Ngoro in Ikwo Local Government Area, Abia State. Dried fifty (50) seeds per genotype were packed in brown labeled envelopes were treated with x-ray radiation at the x-ray unit of Federal Medical Centre (FMC) Umuahia, Abia State, Nigeria with five mutagenic doses (0, 5, 10, 15 and 20 mGy). The experiment was conducted at the Teaching and Research Farm of Michael Okpara University of Agriculture Umudike in the rainforest zone of Nigeria (5⁰.29’N, 7⁰.33’E). The experimental design was a 4x5 factorial experiment in a randomized complete block design (RCBD) with three replicates. The plot size was 2 m x 2 m with 1 m apart. Planting was done at 30 cm intra row spacing and two seed per hole measuring 3-4 cm deep. In addition, four plots were assigned for each sampling unit each containing 40 stands. Observation and measurement on vegetative parameters recorded are: Days to 50% emergence, plant height, number of leaves, leaf area using the constant developed by [17], stem girth and number of branches, Days to 50% flowering, pod length, number of seeds per pod, number of pods per plant, 100 seed weight and grain yield per hectare (t/ha). The collected data on various parameters under study were statistically analyzed using [18]. Analysis and interpretation were based on [19] and the mean separations were carried out using least significant, difference (LSD) at 5% level of significance.

3. Results and Discussion

The observations made on the general morphology of the x-ray irradiation cowpea varieties showed that some were full and bushy possessing dark green foliage with regards to the comparative control. The analysis of variance showed that days to 50% emergency varied among the cowpea varieties studied as shown in Table 1. Where varieties of Black-eyes and Dark brown eye were delayed at days to 50% emergency, Ife-Brown and Light brown-eye were quickened by the effect of the mutagenic doses as they emerged earlier.

Table 1: Effect of mutagenic and variety on two plant characters of Cowpea

Variety	Mutagenic Doses	Days to 50% Emergence
Black Eye	0	3.8
	5	6.3
	10	9.3
	15	4.8
	20	8.8
Dark Brown Eye	0	3.9
	5	9.5
	10	5.2
	15	10.7
	20	10.4
Ife Brown	0	4.6
	5	3.2
	10	9.2
	15	7.7
	20	7.3
Light Brown Eye	0	11.4
	5	11.0
	10	8.5
	15	10.6
	20	8.7
Mean		7.7
LSD _(0.05) for Mutagenic Doses		1.4
LSD _(0.05) for Variety		
LSD _(0.05) for interaction		1.3
Coefficient of Variation (%)		2.8
		17.7

There was significant increase in plant height and number of leaves after x-irradiation as shown in Table 2 and 3. X-ray irradiation beyond 15 mGy, led to a decrease in plant height and number of leaves in all the varieties, whereas mutagenic doses of 10 mGy and 15 mGy increased the two plant characters in all the varieties.

Table 2: Effect of mutagenic doses and variety on plant height (cm) of Cowpea at 2, 4, 6, 8, 10 and 12 Weeks after planting (WAP)

Variety	Mutagenic Doses	Plant Height (cm)					
		2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
Black eye	0	7.0	9.5	21.8	98.9	114.6	135.4
	5	6.7	10.5	23.4	124.5	144.2	170.4
	10	7.3	8.7	32.0	170.4	197.3	233.1
	15	5.2	7.3	25.0	75.0	86.8	102.6
	20	7.1	10.8	21.7	104.7	121.2	143.2
Dark Brown eye	0	8.6	10.3	12.5	71.6	82.9	97.9
	5	8.1	7.7	22.1	122.9	142.3	168.2
	10	7.7	6.6	16.2	81.1	93.8	110.9
	15	6.4	8.1	13.8	149.1	172.7	204.1
	20	7.9	9.2	12.9	105.2	121.8	144.0
Ife Brown	0	8.5	11.4	20.8	110.6	128.1	151.4
	5	7.5	9.2	37.0	65.2	75.5	89.2
	10	8.2	10.1	8.5	131.3	152.0	179.6
	15	8.6	10.0	16.4	148.4	171.8	203.0
	20	6.3	7.0	15.3	61.2	70.9	83.8
Light Brown eye	0	4.8	6.0	31.0	120.6	139.7	165.1
	5	7.0	8.5	15.9	154.2	178.5	211.0
	10	8.3	4.8	31.2	168.9	195.5	231.1
	15	7.5	9.8	9.4	205.3	237.7	280.9
	20	4.8	4.5	26.2	99.6	115.3	136.3
Mean		7.2	8.5	20.7	118.4	137.1	162.1
LSD _(0.05) for Mutagenic Doses		NS	NS	NS	9.4	10.9	12.9
LSD _(0.05) for Variety		NS	NS	NS	8.4	9.8	11.5
LSD _(0.05) for interaction		NS	NS	NS	18.8	21.8	25.8
Coefficient of variation (%)		9.7	8.1	5.3	13.3	13.3	13.4

Table 3: Effect of mutagenic doses and variety on number of leaves of Cowpea at 2, 4, 6, 8, 10 and 12 Weeks after planting (WAP)

Variety	Mutagenic Doses	Number Of Leaves					
		2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
Black eye	0	5.8	13.8	37.8	148.4	197.9	128.6
	5	6.3	18.3	45.1	186.8	249.1	161.9
	10	6.4	14.9	42.6	255.6	340.8	221.5
	15	4.1	14.8	34.2	112.5	150.0	97.5
	20	6.3	17.2	55.5	157.0	209.3	136.1
Dark Brown eye	0	7.7	15.5	45.1	107.4	143.1	93.0
	5	7.7	10.3	21.8	184.4	245.8	159.8
	10	7.7	8.7	33.0	121.6	162.1	105.4
	15	7.1	13.1	32.7	223.7	298.3	193.9
	20	7.3	16.0	33.6	157.8	210.4	136.8
Ife Brown	0	8.1	17.1	62.4	165.9	221.2	143.8
	5	6.7	14.8	34.9	97.8	130.4	84.7
	10	7.5	18.7	44.2	196.9	262.5	170.6
	15	8.2	18.8	57.9	222.6	296.8	192.9
	20	6.2	11.4	27.0	91.9	122.5	79.6

Light Brown eye	0	4.9	12.9	22.6	180.9	241.2	156.8
	5	7.3	17.3	48.3	231.3	308.4	200.4
	10	7.6	8.7	20.3	253.3	337.7	219.5
	15	6.4	19.6	40.1	307.9	410.6	266.9
	20	5.9	9.3	23.1	149.4	199.2	129.5
Mean		6.8	14.6	38.1	177.6	236.9	154.0
LSD _(0.05) for Mutagenic Doses		NS	NS	NS	14.1	18.8	12.2
LSD _(0.05) for Variety		NS	NS	NS	12.6	16.8	10.9
LSD _(0.05) for interaction		NS	NS	NS	28.2	37.7	24.5
Coefficient of variation (%)		5.7	13.6	10.0	13.3	13.3	13.3

Leaf area and number of branches were all significantly increased in all the cowpea varieties. In Black eyed and Ife brown the mutagenic doses of 10 mGy increase the leaf area and the number of branches as compared to the control while the mutagenic dose of 15 mGy increased the number of branches and leaf area in Dark brown eyed and Light brown eyed as shown in Table 4 and 5.

Table 4: Effect of mutagenic doses and variety on leaf area of Cowpea at 2, 4, 6, 8, 10 and 12 Weeks after planting (WAP)

Variety	Mutagenic Doses	LEAF AREA (cm ²)					
		2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
Black eye	0	12.4	13.0	16.3	13.7	13.5	13.8
	5	15.6	16.4	20.5	15.7	12.6	15.7
	10	22.2	23.3	29.2	14.2	11.4	14.2
	15	9.4	9.9	12.3	12.3	15.2	12.4
	20	13.1	13.8	17.2	19.2	15.1	19.4
Dark Brown eye	0	8.9	9.4	11.8	17.2	15.5	17.4
	5	15.4	16.2	20.2	13.2	12.0	13.2
	10	10.1	10.7	13.3	9.8	14.2	10.4
	15	18.6	19.6	24.5	10.9	10.3	11.2
	20	13.1	13.8	17.3	12.6	9.4	12.8
Ife Brown	0	13.8	14.6	18.2	21.8	22.0	22.0
	5	8.1	8.6	10.7	14.0	14.0	14.1
	10	19.5	20.5	25.6	17.0	17.2	17.2
	15	18.5	19.5	24.4	16.8	16.9	17.0
	20	7.7	8.1	10.1	18.3	16.3	13.6
Light Brown eye	0	15.1	15.9	19.8	11.9	10.1	11.9
	5	19.3	20.3	25.4	13.7	14.5	12.1
	10	21.1	22.2	27.8	7.6	10.5	7.8
	15	28.7	30.2	37.7	15.0	12.7	15.3
	20	12.4	13.1	16.4	9.9	13.0	10.1
Mean		15.2	15.9	19.9	14.2	13.8	14.1
LSD _(0.05) for Mutagenic Dose		1.2	1.2	1.5	NS	NS	NS
LSD _(0.05) for Variety		1.0	1.1	1.4	NS	NS	NS
LSD _(0.05) for interaction		2.3	2.5	3.1	NS	NS	NS
Coefficient of variation (%)		15.0	15.0	15.0	8.1	9.8	8.8

Table 5: Effect of mutagenic and variety on Number of branches of Cowpea

Plant	Character	
Variety	Mutagenic Doses	Number of Branches
Black Eye	0	5.0
	5	6.3
	10	8.9
	15	3.8
	20	4.9
Dark Brown Eye	0	3.6
	5	6.2
	10	4.1
	15	7.5
	20	5.3
Ife Brown	0	5.6
	5	3.3
	10	7.9
	15	7.5
	20	3.1
Light Brown Eye	0	6.1
	5	7.8
	10	8.5
	15	11.6
	20	4.9
Mean		6.1
LSD _(0.05) for Mutagenic Doses		0.5
LSD _(0.05) for Variety		0.5
LSD _(0.05) for interaction		1.1
Coefficient of Variation (%)		14.6

The results on Table 6 revealed that cowpea varieties differed significantly on days to 50% flowering. Ife brown at 5 MGy and Light brown-eye at 10 MGy flowered between 38.6 days and 89 days whereas on Black eye and Dark brown eye the x-ray irradiation levels did not show any significant effect.

Also, the pod length (g) and seed yield per pod (g) in all the cowpea varieties were significantly increased at 10 mGy and 15 mGy. The effect of x-ray radiation on number of pods per plant, number of seeds per pod, 100 seeds weight and grain yield per hectare are shown in Table 7. The result showed that there was a significant difference at $P < 0.05$ on all the plant characters in the four varieties studied. Also, the combined effect of interaction between the mutagenic doses and the varieties of the doses 10 mGy and 15 mGy showed a significant difference on the four plant characters in Table 7.

Table 6. Effect of mutagenic doses and variety on two plant characters of Cowpea

Variety	Mutagenic Doses	Characters		
		Days to 50% flowering	Pod length (cm)	Seed yield Per pod (g)
Black eye	0	46.1	10.4	52.4
	5	75.6	13.1	66.0
	10	92.8	17.9	94.0
	15	57.0	7.9	39.7
	20	92.0	11.0	55.4
Dark Brown eye	0	46.8	7.5	37.9
	5	86.9	12.9	65.1
	10	62.3	8.5	42.9
	15	113.1	15.7	79.0
	20	107.5	11.1	55.7
Ife Brown	0	54.8	11.6	58.6
	5	38.6	6.9	34.5
	10	110.3	13.8	82.5
	15	91.8	15.6	78.6
	20	73.4	6.4	32.4
Light Brown eye	0	121.2	12.7	63.9
	5	109.3	16.2	81.7
	10	89.0	17.8	89.4
	15	96.5	21.6	121.5
	20	90.0	10.5	52.7
Mean		82.8	12.5	64.2
LSD _(0.05) for Mutagenic Doses		17.2	1.0	4.9
LSD _(0.05) for Variety		15.4	0.9	4.4
LSD _(0.05) for interaction		34.4	2.0	9.9
Coefficient of variation (%)		12.8	13.3	15.0

Table 7: Effect of mutagenic doses and variety on four yield characters of Cowpea

Variety	Mutagenic Doses	Plant Characters			
		Plant 100 Seed Weight (g)	Number of seeds Per pod	Number of pod Per plant	Grain yield Per hectare (t/ha)
Black eye	0	26.2	6.5	15.0	1.7
	5	33.0	8.2	18.8	2.2
	10	47.0	11.7	26.8	3.0
	15	19.9	4.9	11.3	1.3
	20	27.7	6.9	14.8	1.8
Dark Brown eye	0	19.0	4.7	10.8	1.3
	5	32.5	8.1	18.6	2.2
	10	21.5	5.3	12.3	1.4
	15	39.5	9.8	22.6	2.6
	20	27.9	6.9	15.9	1.9
Ife Brown	0	29.3	7.3	16.7	1.9
	5	17.3	4.3	9.9	1.1
	10	41.3	10.3	23.6	2.3
	15	39.3	9.8	22.5	2.6

	20	16.2	4.0	9.3	1.1
Light Brown eye	0	31.9	7.9	18.3	2.1
	5	40.8	10.1	23.3	2.7
	10	44.7	11.1	25.6	3.0
	15	60.7	15.1	34.7	3.6
	20	26.4	6.6	14.6	1.9
Mean		32.1	8.0	18.3	2.1
LSD _(0.05) for Mutagenic Doses		2.5	0.6	1.6	0.2
LSD _(0.05) for Variety		2.2	0.6	1.4	0.1
LSD _(0.05) for interaction		4.9	1.2	3.2	0.3
Coefficient of variation (%)		15.0	15.0	14.6	13.3

Discussion

The result of this study revealed that x-ray radiation had a significant effect on the vegetative parameters of the cowpea varieties studied. With respect to Days to 50% emergence, the mutagenic doses produce a physiological effect including delayed emergence in comparison to the control as shown in Black-eyed and Dark brown-eyed varieties. This agrees with [20] who reported delayed in germination time with effect on germination percentage on wheat treated with gamma irradiation. Also, [21] has reported effect of higher doses which had negatively influence on germinating of *Datura Innoxia*. The tendency of x-ray radiated seeds of the varieties of Ife Brown and Light brown-eyed to emerge between 3-8 days in relation to the control suggest that x-ray irradiation doses induced increased enzymatic activities, which could have initiated the early germination and seedling survival. This has been reported by [22] who opined that germination and seedling survival of *Cumminum cyminum* improved at a lower dose (100Gy). [23] has also showed that dose range of 0.5-5 kR can be used as an effective treatment for enhancing germination of sunflower. The increase in plant height revealed significant impact of the mutagenic doses on the cowpea varieties as distinguished with the control. However, doses (5 mGy, 10 mGy and 15 mGy) were more effective than higher dose (20 mGy) as noticed at various weeks of data collection which favored more branching as a determining factor of yield. This observation agrees with the work of [24] who reported occurrence in reduction in plant height with higher doses of gamma rays, which is characterized by gross injury caused at cellular level. The x-ray doses of 10 mGy and 15 mGy increased the number of branches among the four varieties of cowpea compared to the control. This also has been demonstrated by [25] that 80 kR radiation doses increased the number of branches and stem diameter of medicinal plant *Sophora davidii*. With respect to the vegetative parameters of four cowpea genotypes, lower x-ray radiation treatments (10 mGy and 15 mGy) increased the plant height, stem girth, number of branches, leaf area and number of leaves. This indicated that there exists some stimulating effect of x-ray radiation from 10 mGy-15 mGy on the cowpea genotypes based on the plant vigor. Also, the significant plant vigor as initiated by the x-ray mutagens at 10 mGy-15 mGy in all the four cowpea genotypes as compared to the control might be due to chromosomal aberration that tend to produce an increase in definite morphological traits such as plant height, number of leaves number of branches stem girth. Similar observations were reported with low dose irradiation up to 100Gy of gamma irradiation on three cowpea varieties [26]. [27] have also reported that low radiation

doses are accompanied by early emergency, increased percentage germination and field survival. [28] also reported that increase in gamma ray doses and sodium azide concentrations also increase leaf number in pigeon pea. There was increase in leaf area and stem girth of the four cowpea varieties as compared to the control. This revealed that x-ray radiation doses of 10 mGy and 15 mGy increased the leaf area and stem girth compared to the control. [29] reported increase in leaf area when pre-germination radiation treatment (100Gy) was applied to cowpea. This result is also in agreement with [30] who reported that shoot length stem diameter, leaf length and width of *Abelmoschus esculentus* were significantly increased at radiation dose of 400Gy when compared to non-irradiated plants. The definite period in a given plant life is the fruiting point because it is used as an anticipatory factor towards fruiting and yielding capability of crop plants. The result revealed that x-ray irradiated seeds of Dark brown eye and Black eye varieties required more days to flower compared to the control. This agrees with the work of [26] who reported that gamma irradiation showed no marked improvement in number of days to flowering. All the irradiated plants required more days to flower than the control. However, in Ife brown and Light brown eye varieties, the results showed that the mutagenic doses of 5 mGy and 10 mGy x-ray irradiation quickened flowering days on the varieties as compared with the control. Also [26] have reported similar finding which showed that low dose of 5 Gy of gamma ray irradiation caused early flowering in some cowpea varieties. This divergence could be as a result of some changes in environmental phenomena like temperature, sunlight and rainfall. The positive increase in the pod length and number of seeds per pod revealed that in all the four cowpea varieties mutagenic doses (10 mGy and 15 mGy) were significant. Also increase in the length of pod by the mutagenic dose indicates good pod filling and enhance cowpea productivity which reflect the number of seed per number of seeds per pod. Similar observation of increase number of seeds per pod have been reported by [26], [28], [31] and [32]. The increase in 100 seed weight and grain yield per hectare in the four cowpea varieties by the x-ray irradiation doses (10 mGy and 15 mGy) might have stimulated increase in plant characters. Such increase was also reported by Ogidi *et al.* (2010) that gamma ray of 10-15 KR increased 100 – seed weight (g) of some cowpea varieties. This also correspond with the reports of [33] and [34] (2014) who had earlier reported increase in yield of crops treated with mutagens.

4.0 Conclusion

To increase possibility of inducing specific type of genetic variation in the crop (cowpea), x-ray irradiation doses of 10-15 mGy which constantly produced the optimal effects on the crop should be adopted. This study has revealed that ionizing radiation such as x-ray irradiation could be used to generate genetic variability for breeding improved plants and for the purpose of genetic studies in cowpea.

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