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ORIGINAL ARTICLE

EFFECT OF X-RAY RADIATION ON EMERGENCE AND SEEDLING GROWTH IN COWPEA (Vigna unguiculata (L) Walp) BORNO BROWN Var. IN SUDAN SAVANNA OF NIGERIA

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Abstract

Cowpea seeds (variety: Borno Brown) were exposed to five different doses of X-ray radiation (5, 10, 15, 20 and 25kr) and a check (0kr) making a total of six treatments, which were evaluated in a completely randomized design replicated four times. Screen house trials were conducted during 2018(M_1) and 2019(M_2) dry season in the Screen House of Faculty of Agriculture, University of Maiduguri, Borno State. The study was carried out to determine the genetic variation on emergence and seedling growth in Cowpea (Borno Brown) by the action of a physical mutagenic agent (X-ray radiation). Evaluations were also done to estimate the types and frequency of mutant phenotypes observed in M_1 and M_2 trials. Results from the analysis of variance showed that there were significant differences ($p<0.05$) in the level of radiation in the characters evaluated. However, the cultivar produced almost the same spectra to mutant but with different frequencies. Seed germination decreased (14-12m_1, 15-10m_2) as the level of radiation increased and seedling survival (M_1M_2) depended very much on the frequency of lethality (20-25kr) of X-ray radiation to the plant in the population. The appearance of leaf spots (1.83), alteration in the leaflet number of normal trifoliate leaves and distortions at early growth (4, 0.3 and 0.17) appeared to be related to X-ray radiations, since effects were absent in the control (0). These effects can appear at various stages of plant development and may cause abnormal cell division, cell death, mutation, tissue and organ failure and reduction of plant growth. Therefore, there is need for more evaluation in the radiation doses in other to get more mutants of economic importance towards sustainable crop production. Key words: Mutation, Cowpea, Cell, Screen house.

Introduction

Cowpea (Vigna unguiculata (L.)Walp) belongs to the family Leguminosae and sub-family Fabaceae, having chromosome number 22, it is herbaceous annual crop commonly referred to as bean, black-eye pea, kafir pea, Southern pea, China pea, marble pea, lubia, niebe, coupe or frijole (Olowe, 1981). It also includes vegetable types such as yard long bean and asparagus. Cowpea originated in Africa and it became an integral part of the traditional cropping system throughout Africa, particularly in the semiarid region of West Africa savannah. Davies et al. (2005) and Jefferson (2005) attested that cowpea is an ancient crop whose cultivation began in Africa between 5,000 and 6,000 years ago. Cowpea plays an important role in providing soil nitrogen to cereal crops (such as maize, millet, and sorghum) when grown in rotation, especially in areas where poor soil fertility is a problem. It does not require a high rate of nitrogen fertilization; its roots have nodules in which soil bacteria called rhizobia to inhabit and help to fix nitrogen from the air into the soil in the form of nitrates (Sheahan, 2012).

Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant in various fields like morphology, cytogenetics, biotechnology and molecular biology (Khan et al., 2005). Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Geeta et al., 2011) and an efficient means of supplementing existing germplasm for cultivar improvement in breeding programs. Mutation is a sudden permanent heritable change in an organism gene. It is produced by the change in the base sequence of genes and it can be induced both in seed and vegetatively propagated crops. Induced mutations have recently become the subject of biotechnology and molecular investigation leading to the description of the structure and function of related genes. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Khan et al., 2005).

X-rays belong to physical ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect the morphology, anatomy, biochemistry and physiology of plants differentially depending on the irradiation level (Bhatt et al., 2001).

Borno brown cowpea has been found to be the most widely used and cultivated cowpea variety in Sudan savannah. But due to the continuous use of local and genetically unimproved variety, low yield, disease and pest infestation is estimated to still be on the increase in important cowpea growing regions of the study area. This research is aim to alleviate these problems through the induction of mutation which is usually resorted to create variability not available in the gene pool or to correct specific deficiency of an otherwise outstanding genotype. Induced mutagenesis is one of the alternative breeding methods, which can be applied to enhance the variability and to correct one or more defects (low yield, disease and pest infestation etc.) in the variety.

Materials and Methods

Experimental Site

Screen house trials were conducted during the 2018(M_1) and 2019(M_2) dry season in the Screen House of Faculty of Agriculture, University of Maiduguri, Borno State, in the Sudan Savanna agro-ecological zone of Nigeria, with latitude and longitude of 11^0 48'40'N and 13^0 12'23'E, respectively.

Experimental design

The research involves cowpea seeds variety (Borno Brown) predominantly grown by the farmers in the experiment area, that were exposed to five different doses of X-ray radiation (5, 10, 15, 20 and 25 kr) and check (0kr) making a total of six treatments. X-ray radiation was used as the mutagenic agent during this experiment. The radiation process was carried out at department of Radiography, Yobe State University Teaching Hospital, Damaturu. Four seeds were sown in plastic pots of 32 cm in length and 22 cm in diameter, which were evaluated in a Completely Randomized Design (CRD) replicated four times. Data were collected on seed germination base on the number of seeds which emerged, Seedling survival was on the number of plants which become established out of the total number of seedlings that emerged in each treatment, variations were observed and compared between treatments and control in both $M_1$and M_2 generations. Seedlings with deviating also recorded. Phenotypic mutants obtained in each level of radiation were grouped according to types. Mutation frequencies were calculated as the percentage of mutant plant to normal individuals for each level of treatment (5, 10, 15, 20 and 25 kr). All data collected were subjected to analysis of variance (ANOVA) and the means were compared by LSD at 5% level of probability.

Results and Discussion

Effect of X-Ray Radiation on Seedling Emergence and Seedling Survival in M_1 Generation (2018) and M_2 Generation (2019).

The result on the effect of X-ray radiation in M_1 and M_2 generations on seedlings emergence and seedling survival are presented in Table.1. For seedlings emergence, M_1 and M_2 results indicated that significant differences ($p<0.05$) were observed among the treatments, where 5 and 10kr showed the highest number (14) of seedling emerged among the treatments, while 20 and 25kr exhibited the least number (12) of seed emerged. It was observed that the rate of germination decreased (14-12m_1, 15-10m_2) as the dose of the X-ray radiation increases, implying that higher (14) rate of germination was obtained at lower levels of exposure, than at higher doses which causes chromosomal aberration and eventually killed the cotyledon cells. This indicates that the mutagen is effective in inducing genetic variability in cowpea. Similar result was reported by Bhosle and Kothekar (2010). This is also in conformity with the findings of (Mandal et al. 2007), where significant statistical differences were recorded among the mutants in M_1 trial using gamma rays on groundnut.

Similarly, on seedling survival, significant differences ($p<0.05$) were recorded in both trials (M_1 and M_2), where 5kr and 10kr produced the highest number of seedlings survival (14). The number of seedlings (14) that survived seemed to depend on the number of lethal phenotypes at the seedling stage. The decrease in effectiveness at higher dose treatments may be ascribed to the failure in proportional increase in mutation frequency with increase in dose of the mutagens. Similar observation was made by Konzak et al., (1965), using chemical mutagenesis and further stated that the higher efficiency at lower and intermediate doses of mutagens as observed in the study, might be due to the fact that the biological damage (lethality and sterility), increased with an increase in dose at a rate greater to the frequency of mutations. This finding is also in accordance with the earlier reports of Nawale et al, (2006), Ugorji et al. (2012), Dhanavel and Girija (2009).

On germination and survival percentages, high number of seedlings (93.8-75.0%m_1, 99.962.5%m_2) emergence were recorded at low radiated treatments in both M_1 and M_2 generations where 5kr showed the highest germination percentage. Similarly, on survival percentages, treatment 5kr recorded the highest survival percentage (87.50%) in M_1 and M_2 generations. The low percentage seedling emergence and very low or no seedling survival observed at higher doses were probably due to severe damage of some vital embryonic cells or tissues. Lagoda (2012) and Mudibu et al. (2012) among many authors reported that gamma (ionizing) radiation can damage and affect the morphology, anatomy, physiological and biochemical processes in plants depending on the radiation level. These effects can appear at various stages of plant development and may cause abnormal cell division, cell death, mutation, tissue and organ failure and reduction of plant growth.

Table 1: Effect of X-Ray Radiation on Seedling Emergence and Seedling Survival in M_1 Generation (2018) and M_2 Generation (2019)

Treatment X-ray rad (kr)	No. of seeds planted	Total no. of seeds Emerged		edlings rvival		Emergence		Survival	
		M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
0	16	15^{a}	16^{a}	15^{a}	15^{a}	93.75^{a}	99.90^{a}	93.75^{a}	93.75^{a}
5	16	14^{a}	15^{a}	14^{a}	14^{a}	87.50^{b}	93.75^{b}	87.50^{b}	87.50^{b}
10	16	14^{a}	15^{a}	13^{a}	12_{ab}	87.50^{b}	93.75^{b}	81.25^{c}	75.50^{c}
15	16	12^{b}	13_{ab}	12^{b}	10^{b}	75.00^{d}	81.25^{d}	75.00^{d}	62.50^{d}
20	16	13^{a}	10^{b}	12^{b}	8_{b}	81.25^{c}	62.50^{c}	75.00^{d}	50.00^{e}
25	16	12^{b}	10^{b}	12^{b}	7_{bc}	75.00^{d}	62.50^{c}	75.00^{d}	43.75^{f}
Mean	16	13	13	13	11	83.33	82.26	81.25	68.83
SE±	NS	0.49	1.07	0.51	1.32	3.09	6.77	3.23	8.24

Means in the same Column followed by the same letter(s) are statistically similar at 5 % level of probability according to DMRT; NS: Not significant

Types and Frequencies of Mutant Observed in M_1 Generation (2018) and M_2 Generation (2019)

Table 2 and 3 show the result on types and frequencies of mutants observed in M_1 and M_2 trials Yellow seedlings mutants; significant differences ($p<0.05$) were observed among the treatments in M_1 and M_2 trials, where treatments 15kr, 20kr and 25kr showed significant difference when compared with the remaining treatments in M_1 trials, while 25kr showed the highest number (4) of yellow seedling mutants in M_2 trials. These appeared as bright yellow seedlings devoid of chlorophyll in their primary leaf, cotyledon and stems which some were reverted to normal green and grew to maturity. The yellow or white seedlings were lethal mutants, because they were devoid of chlorophyll needed for photosynthesis. Olasupo (2004) reported similar observations from cowpea seeds treated with ethyl methane sulphonate. Abnormal leaves mutant; significant differences was not observed among the treated cowpea seeds (5kr, 10kr, 15kr, 20kr and 25kr) except for control (0kr) which recorded no abnormal leaves mutant (0) in M_1 generation. While M_2 trials showed significant differences among the treated cowpea seeds where 25kr recorded the highest number(5) of abnormal leaves mutants. Leaf variegation is a common mutation that can be either a nuclear or cytoplasmic mutation. Gunckel and Sparrow (1961) explained that the leaf abnormalities might be attributed to chromosomal breakage, disrupted auxin synthesis and transport, disruption of mineral metabolism. Others were observed with notable variation in shape, size, number and arrangement of leaflets with various mutagenic treatments. The results are consistent with earlier research reported by Horn and Shimelis (2013) in cowpea, Mudibu, et al. (2012) in soybeans, Harding et al. (2012) in rice, Kon et al. (2007) in long beans and Norfadzrin et al. (2007) in tomato and okra.

For erect types mutants, the result showed significant differences among the treatments in both M_1 and M_2 generations. Where only 15kr and 25kr treatments exhibited erect types mutants in M_1 generation, while in M_2 trials, 25kr produce the highest number (3) of erect type's mutants. These mutants exhibited upright growth, increase height (tall), branches with elongated leaves and long internodes. This was also reported by Kumar et al. (2009) in black gram, Solanki et al. (2004), Khursheed and Khan (2014) in lentil.

For dwarf plant mutant, which exhibited small internodes and decreased number of branches which might be due to decrease in cell division. The result showed significant differences among the treatments in both M_1 and M_2 trials, where only 20kr treatment produced dwarf plant mutants in M_1 generation while 20kr and 25kr treatments recorded the highest number (4 and 2) of dwarf plant mutant in M_2 trials. It was reported by Hedens (2003) that reduction in plant height was due to alteration in gibberellic acid whereas, Kleinhofs et al. (1978), Suganthy et al. (1994) reported that decrease in plant height was due to mitotic irregularities. Konzak et al. (1969) in Triticum and Shakoor et al. (1978) in triticale revealed that semidwarf characteristic was regulated by polygenes.

For male sterile mutant, which were characterized by their inability to produce functional pollen, hence self-pollination could not occur. The flower dried and later dropped thereby preventing the flower to produce fruit. Significant differences ($p<0.05$) were observed among the treatments in both trials (M_1 and M_2), where only 25kr treatment produced a sterile plant mutant (1) in M_1 generation while 20kr treatment showed the highest number (4) of sterile mutants in M_2 generation. However, an earlier finding confirms that pods can be formed when they are crossed with normal fertile plants, and these sterile mutants were found to be moderately resistant to aphids. This will enable the creation of additional genetic diversity by inducing mutation to supplement existing variability for cowpea improvement. These results agree with the report of Forster and Shu (2012) that improvement in plant breeding can only be made when sufficient variation for a given trait is available to the breeder.

On mutational frequency, 25kr recorded the highest mutational frequencies (50 and 100) in both M_1 and M_2 generation, where 10kr in M_1 and 5kr M_2 showed the least mutational frequencies among the X-ray treated cowpea seeds.

The present study demonstrated that most characters of cowpea which are of interest to plant breeders can be altered through mutations using the X-ray irradiation technique. Furthermore, new plant attributes were created in the high yielding and well adapted local cowpea varieties.

Table 2: Types and Frequencies of Mutant Observed in M_1 Generation (2018)

X-ray rad (Kr)	No. of seeds planted	No. of seeds emerged	Yellow seedlings	Abnormal leaves	Erect type	Dwarf	Sterile plants	Mutational frequency
0	16	15^a	0_b	2_b	0_b	0_b	0_b	12.50
5	16	14^a	0_b	5_a	0_b	0_b	0_b	31.25
10	16	14^a	0_b	4_a	0_b	0_b	0_b	25.00
15	16	12^b	1_a	5_a	1_a	0_b	0_b	43.75
20	16	13^a	1_a	3_a	0_b	1_a	0_b	31.25
25	16	12^b	1_a	5_a	1_a	0_b	1_a	50.00
Mean	16	13	1.83	4	0.3	0.17	0.17	32.29
SE±	NS	0.49	0.22	0.51	0.21	0.16	0.16	5.45

Means followed in the same Colum by the same letter(s) are statistically similar at 5 % level of probability according to DMRT; NS: Not significant

Table 3: Types and Frequencies of Mutant Observed in M_2 Generation (2019)

X-ray rad (Kr)	No. of seeds planted	No. of seeds emerged	Yellow seedlings	Abnormal leaves	Erect type	Dwarf	Sterile plants	Mutational frequency (%)
0	16	16^a	0_d	0_d	0_d	0_c	0_d	0.00
5	16	15^a	1_c	0_d	0_d	0_c	0_d	6.25
10	16	15^a	1_c	1_c	1_c	0_c	1_c	25.0
15	16	13^b	2_b	3_b	1_c	1_b	1_c	50.0
20	16	10^c	3_a	3_b	2_b	2_a	4_a	87.5
25	16	10^c	4_a	5_a	3_a	2_a	2_b	100.0
Mean	16	13	1.83	2	1.17	0.83	1.3	44.79
SE±	NS	1.07	0.60	0.81	0.47	0.40	0.61	17.10

Means followed in the same Column by the same letter(s) are statistically similar at 5 % level of probability according to DMRT; NS: Not significant

Conclusion

From the study, it can be concluded that there were significant differences that occurred among the treatments for most of the characters studied which indicates the presence of genetic variability among these genotypes which could be exploited to enhance selection for further improvement in cowpea. The study identifies that relatively low radiated treatments (5 and 10kr) were more consistent based on the characters determined. Highly radiated treatments were lethal and can cause damage to the cells which affect the morphology, anatomy, physiological and biochemical processes in plants that lead to abnormal cell division, cell death, mutation, tissue and organ failure and reduction of plant growth. The rate of mutation recorded in M_1 and M_2 generations shows that the mutation frequencies increases as the level of radiation increased. Treatments 15kr, 20kr and 25kr exhibit the highest mutational frequency and produced more type of mutant plants. These mutants may be useful in cowpea population improvement programs to facilitate outcrossing in recombination cycles.

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