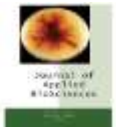

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Effect of gamma rays on some qualitative traits of three genotypes of Okra (*Abelmoschus esculentus* (L.) Moench) in Burkina Faso

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ABSTRACT

Objectives: The objective of this study is to evaluate the effects of eleven doses of gamma rays on the qualitative phenotypic traits of three okra genotypes, UAE22, KBG535 and KBG24, from Burkina Faso, in order to initiate mutation breeding. Plants with valuable traits will be grown in further generations for selection purpose.

Methodology and Results: Two hundred and fifty (250) seeds of each genotype were irradiated by 60Co sources at doses ranging from 200 to 2000Gy. Seeds were sown in pots and plants grown at two per pot. Third leaf narrowing, stem ramification and plant creeping aspect were accentuated from 200 to 1800Gy. Various colours, shapes and positions of fruits were recorded between 200 and 1400Gy. At 500 and 600Gy, yellowish green fruits with dark green edges for KBG535 and KBG24 and two fruits at the same node were observed for the 3 genotypes. At 500Gy, KBG535 showed an atypical fruit with a twisted end.

Conclusions and application of findings: The characteristics studied showed variation depending on irradiation doses and genotypes. KbG535 exhibited the greatest variability for fruit shape and colour with the tendril shape of the fruit tip at 500Gy and the dark green stripes of the fruit edges at 500 and 600Gy. This tendril shape was not shown in okra descriptors. The pattern of two fruits at the same node and stem ramification with the level of dose may be used to improve yield for all 3 genotypes. In addition, the various colours observed are a proof of possibility to meet consumer needs, since fruit colour is a preferred trait for okra. The irradiation of okra seeds is therefore a source of variability in qualitative traits. Additional experiments in further generations are however necessary to assess the heritable nature of this variation.

Keywords: Burkina Faso, irradiation, okra, variability.

INTRODUCTION

Okra (*Abelmoschus esculentus (L.) Moench*) is a food plant of the Malvaceae family. It is eaten as a fruit and leaf vegetable, fresh or dried and is the main vegetable of people living in the city in Burkina Faso (Sawadogo *et al.*, 2009). The genetic diversity of okra is low (Ouédraogo *et al*., 2016) which limits selection and varietal creation. This might be one of the reasons of the lack of improved varieties in Burkina Faso. Geographic distances and environmental differences are the two main causes of genetic diversity in plant populations (Slatkin, 1987; Nosil *et al.*, 2009). Evolutionary forces such as selection, mutation, migration and genetic drift underlie the genetic diversity of crops (Bhandari *et al.*, 2017). The variability created by radiation-induced mutagenesis could help meet the challenge of creating high-performance lines. Indeed, radiations have proven to be the best tools to induce genetic variability within a very short span of time (Pushparajan *et al.*, 2014) and have been the most frequently used method for directly developed of mutant varieties (Lagoda *et al*., 2009). If characters relating to yield improvement are sought, okra is also appreciated for its qualitative morphological characteristics, which determine the preference of farmers for some varieties (Ouédraogo, 2016). Its qualitative traits, namely shape, colour and other quality factors like pubescence are a determining factor in consumer preference (Nesru *et al.*, 2021). Through the induction of a variation linked to qualitative traits, the involvement of radiation-induced mutagenesis in Burkina Faso, in a varietal improvement program for okra, could increase its level of genetic diversity and meet the needs of users. Indeed, the quantitative determination of M1 injury and radiosensitivity testing should be routine procedures in mutation breeding programmes (Spencer *et al.*, 2020) and improvements in plant breeding can only be made when sufficient variation for a given trait is available to the breeder (Forster & Shu, 2011). The success of a mutation breeding program depends on the effectiveness and efficiency of the mutagen used (Arisha *et al.*, 2015). Gamma rays meet these criteria and are available in Burkina Faso. These conditions constitute an opportunity to improve and better enhance the crop because the increase in yield is not sufficient for a fruit vegetable like okra, which is especially appreciated for its qualitative characteristics. A diversity of qualitative characteristics would be an asset for okra producers and sellers with regard to the varied preferences of consumers (Ouedraogo, 2016) and the demands of the national and international market. The aim of this study is to evaluate the effects of eleven doses of gamma rays on the qualitative phenotypic traits of the M1 generation of three okra genotypes from Burkina Faso, in order to perform selection in further generations.

MATERIALS AND METHODS

Plant material: The plant material was three (03) genotypes of okra, including two (KbG535 and KbG24) selected at the “Institut de l’Environment et de Recherches agricoles” (INERA) and one (UAE22) selected at the University Joseph KI-ZERBO, Burkina Faso. These genotypes were chosen based on their agronomic characteristics.

Table 1: Plant material studied

Genotype	Local name	Characteristics
UAE22	Maan kienga	Green and glabrous leaf, single orthotropic green stem, green and slender fruit
KbG535	Kwanabiébié	Green and glabrous leaf, single orthotropic green stem, yellowish-green and slightly tapered fruit
KbG24	Imaani	Green and glabrous leaf with red veins, single orthotropic green stem, green and slightly tapered fruit

(Source: Ilboudo, 2017)

Irradiation protocol: The seeds were irradiated, in August 2020, using the irradiator of the IBD-CETT insectarium in Darsalamy, which is equipped with 2 stationary gamma sources of 60Co. Each source delivered a dose rate of 38.34Gy/min, in the cylinder of suitable geometry, containing the seeds. Due to the pioneering nature of this experiment in Burkina Faso on okra, a wide range of doses was used, namely doses of 200, 400, 500, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000Gy.

Experiment in pots: Seeds irradiated at the different doses were sown in pots containing heat-sterilized soil, at the "Plant Protection" facilities in Bobo-Dioulasso. Each dose-by-genotype treatment was represented by one pot in 3 replicates, including the non-irradiated control treatment. Six seeds were sown and the plants were thinned to no more than 2 plants per pot. The watering of the pots and the weeding by uprooting were done on request.

Data collection and analysis: Data were collected on M1 plants from the 7th DAS onwards, based on visual observations. Each plant was considered as an individual due to the random nature of mutations. Most of the characteristics measured were therefore assessed on all the individuals. The seeds of the M1 plants were collected in order to constitute the progeny, namely the M2 generation.

RESULTS

Observations revealed an induced variation of the qualitative characteristics of the three okra genotypes. The variability of the morphological descriptors of the three genotypes according to the dose was recorded.

Shape of the first true leaf (3rd leaf): For all three genotypes, a narrowing of the 1st true leaf limbus, after the two cotyledon leaves, was observed at 25DAS (figure 1). This narrowing started to be noticeable at 600Gy for KBG24 and KBG535 then at 800Gy for UAE22, and increased with the dose until the said leaf disappeared at high doses, leaving only the cotyledon leaves. For doses less than or equal to 1000Gy for KBG24 and KBG535, and 1400Gy for UAE22, new leaves appeared at 25DAS.

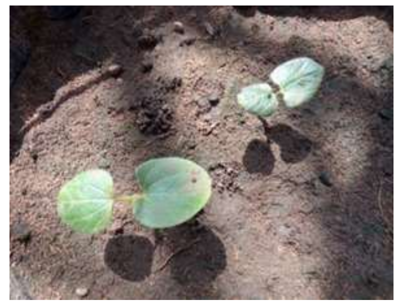
UAE 22	KBG535	KBG24
		
1400Gy: 3rd leaf narrowed till the main nerve and appearance of new leaves	1000Gy: 3rd leaf narrowed till the main nerve and appearance of new wrinkled leaves	1000Gy: 3rd leaf narrowed till the main nerve and appearance of new leaves but wrinkled
		
1800Gy: disappearance of the 3rd leaf and new leaves	1600Gy: disappearance of the 3rd leaf and new leaves	1600Gy: disappearance of the 3rd leaf and new leaves

Figure 1: Gradual narrowing of 3rd leaf limbus until disappearance (25DAS)

Stem branching and plant height aspect: Plant stem branching (figure 2) was observed at 60DAS as a function of dose and genotype. Two branches ramification started at 400Gy and was observed on all subsequent doses. The ramifications with 3 branches alternated or not, were observed from 600Gy and the ramifications with a number of branches greater than 3 concerned only UAE22 from 1400Gy. Indeed, different terms applied. Two branches in Y with parallel stems paired over a certain distance or without pairing of stems; three branches directly separated at the basis, or alternate simple or alternatively with paired stems over a certain distance for each branch and with entanglement of leaf petioles starting almost from the same node; three branches with paired stems of the same basis (spatula shape); more than 3 alternate or paired branches, or 2 separate branches with a tuft of branches at the top of each.

400Gy UAE22: 2 branches paired

800Gy KBG535: 2 branches separated from the basis (Y)

600Gy KBG24: 3 branches paired and entanglement of petioles

1000Gy UAE22: 3 branches united (spatula shape)

1000Gy KBG535: 3 branches separated at the basis

800Gy KBG24: tuft of branches

Figure 2: Examples of stem branching of the 3 genotypes in relation with the dose

In addition, from 800Gy, a significant reduction in the size of the plants (figure 3) was observed and was accentuated with the increase in the dose for the 3 genotypes. The stem slumped to the ground and subsequently straightened towards the top, thus presenting a creeping appearance.

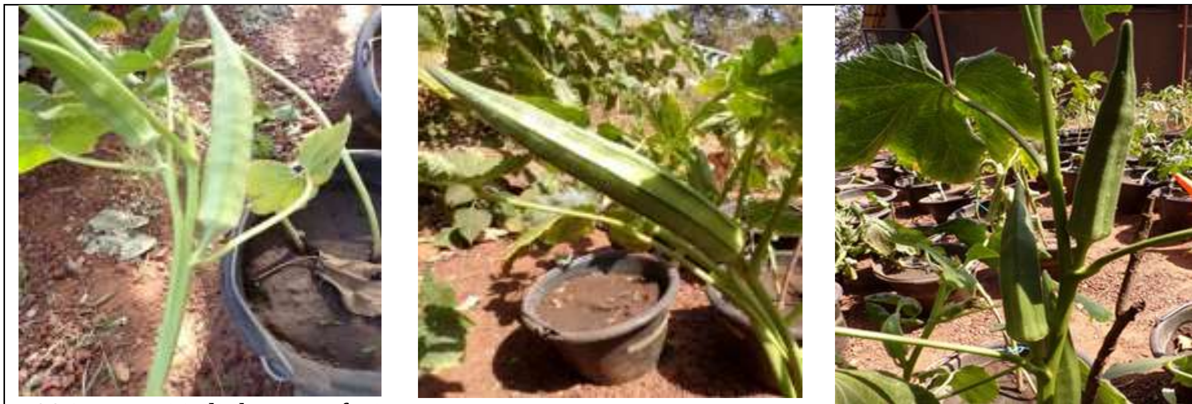
1400Gy UAE22 (60JAS)

1200Gy KBG535 (60JAS)

1200Gy KBG24 (60JAS)

Figure 3: Size reduction and creeping aspect of the plants, dose and genotype related

Fruit colour and shape: The fruit colour of the 3 non-irradiated genotypes was light green at 70DAS. Two other colours were observed after irradiation in KbG535 and KbG24: dark green at 200Gy and yellowish green with dark green edges at 500 and 600Gy. The fruits of UAE22 showed no apparent colour change (figure 4).



400Gy UAE22: light green fruit | 500Gy KBG535: streaked green fruit | 500Gy KBG24: dark green fruit

Figure 4: Examples of fruit colour noted at different doses for the 3 genotypes

In addition, various shapes of the fruit (figure 5) different from that of the control (erected fruit) were observed, especially at doses ranging from 200Gy to 800Gy. They were slightly curved fruits (400Gy on KBG24, 600Gy on KBG535, 800Gy on UAE22), curved fruits (500Gy on UAE22), strongly curved fruits (400Gy on UAE22) and one twisted fruit in the upper half (500Gy on KBG35). However, a trend of variation in shape, associated with an increase or a decrease of doses, was not clearly recorded.

400Gy UAE22: strongly curved fruit | 500Gy KBG535: twisted fruit | 200Gy KBG24: slightly curved fruit

Figure 5: Examples of various fruit shape noted at different doses for the 3 genotypes

Position of the fruits on the stem: Variation was observed at 70DAS in all genotypes according to the doses. It concerned the internode size, the number and the position of the fruits on a node (figure 6). The fruits of the controls had an alternate position on the nodes distant from each other. With increasing dose, and depending on genotype, the following patterns were observed at doses ranging from 200 to 800Gy:

- Insertion of two fruits at the same first node on a single stem, juxtaposed; Insertion of a first fruit on a single stem before branching, then branching with one fruit per branch at the first node of each; ramification with one fruit per branch at the first node of each; two alternated fruits then 2 fruits at the same node on a single stem; three alternated fruits followed by branching without fruit. These results indicated a diversity of fruit positions on the stem, combined or not with branching, depending on the dose.


400Gy UAE22: 2 fruits opposed


500Gy KBG535: branching with 1 fruit per branch at first node


200Gy KBG24: 2 fruits juxtaposed


800Gy UAE22: fruit on single stem, then branching with 1 fruit per branch


600Gy KBG535: 2 fruits juxtaposed


400Gy KBG24: 3 alternated fruits before branching

Figure 6: Examples of position of fruits and branching according to doses and genotypes

DISCUSSION

Effect of radiation dose increase was manifested by the narrowing of the third leaf, well-marked from 600Gy for KBG535 and KBG24, and 800Gy for UAE22. This finally compromised the survival of the plant in preventing the appearance of new leaves or, at best, inducing branching of the stem. However, the dose from which the new leaves are not present at 25DAS differed from one genotype to another. It was higher (800Gy) for the UAE22 genotype than for KBG535 and KBG24 genotypes (600Gy). This testified both to the influence of the dose on the growth of the plant and to a certain resistance of the genotypes to the lower doses. The visible manifestation suggested a high probability of mutation at the doses concerned, in case of survival. Indeed, according to Cooper *et al.* (2008), an increase in mutation frequency can *drastically* reduce the possibility of recovering viable seeds. This study results are similar to those of Kalyani & More (2019), on *Phaseolus lunatus* irradiated at doses of 240 to 420Gy and those of Devmani & Dwivedi (2014) on *Vigna unguiculata* irradiated at doses of 100 to 500Gy, which reported significant damage on the seedlings increasingly with the dose. Indeed, gamma rays were the most destructive mutagen there, compared to ethyl methane sulfonate (EMS) and the EMS-gamma combination. Okra stem branching was observed with dose-dependent intensity. These results are corroborated by Dubey *et al.* (2007) and Sanoop & Udayan (2017) who found, with different doses of gamma rays, an increase in the number of branches per plant. In addition, Dhankhar and Dhankhar (2003) noticed that some plants from seeds of a purple stem line of okra when subjected to gamma irradiation at 0.6 or 0.7kR exhibited flat and Y-shaped branching. In some cases, the main shoot bifurcated in 2 parallel branches, and these parallel branches divided further, repeating the Y-shaped branching pattern. Similar results were obtained by Aaron *et al.* (2017) who observed the Y shape of the stem at 400Gy and multiple ramifications at 600, 800 and 1000Gy. Vinod & Mishra (1999) also noticed the peculiar Y-shaped branching in okra plants derived from seeds irradiated with 400 and 600Gy doses. These results suggest that the branching probability of okra stem from irradiated seeds increases with dose. This branching, if it led to viable stems or the formation of nodes, could increase plant yield. Indeed, a positive correlation of the number of fruits per plant with branching on the one hand and on the other hand with the number of nodes was reported by Ouédraogo (2016). The extent of third leaf limbus narrowing and the likelihood of stem branching seemed to be related. With regard to the plant height aspect, which has been reduced to a dwarf or creeping pattern, the influence of irradiation on the dynamics of plant growth was obvious. This is revealed by the reduction in the plant's height and the length of the internodes, so that prostrate variants occurred. Irradiation at certain doses would inhibit the synthesis of carbohydrates that promote the growth of vegetative organs. Norfadzrin *et al.* (2007) as well as Manju & Gopimony (2009) reported that the growth rate was reduced by the mutagen, especially gamma rays. Brunner (1995) specified that doses of 600-850 Gy would cause a 50% reduction in seedling or epicotyl height of okra. Finally, for Mahamune & Kothekar (2012), it was obvious that the reduction in seedlings height of the varieties, Varun and Waghya, of *Phaseolus vulgaris*, could have been due to significant damage caused at the cellular level, to a decrease of mitotic cell division, to chromosomic alterations or loss of proliferative capacity. These results could explain the decrease in the okra plants height noticed in this study, leading to prostrate or dwarf pattern at high doses. Different colours and shapes of fruits were seen for the 3 genotypes and in relation with doses. Although, a variation of shape according to dose variation was not clearly noticed. The twisted shape of the fruit noticed 500Gy in KBG535 has not been reported in the okra descriptors edited by Charrier (1983). This clearly highlighted gamma radiation effect on okra fruit shape, but heritability of this effect has to be checked. This study results showed diversity in fruits positions on the stem, combined or not with branching in line with the dose. Shortness of internodes until position of two fruits at the same node has also been reported by Sanoop & Udayan (2017). They stated that plants grown from irradiated seeds showed abnormalities such as two fruits at the same node. These data allow expected fruits yield increasing pattern if good agricultural conditions are guaranteed. Characteristics that underwent obvious variation and are correlated with genotypes yield improvement are branching and more than one fruit per node. Then, 3rd leaf limbus narrowing was the early qualitative effect of doses on the plants. Differences within doses effects could be attributed to varietal differences and experimental conditions. All the seeds might not be necessarily uniformly irradiated or not undergo the influence of irradiation with the same magnitude.

CONCLUSION AND APPLICATION OF RESULTS

Irradiation with gamma rays caused in the 3 genotypes of okra from Burkina Faso, a variation of all the qualitative characteristics studied. However, the variations observed were related to both genotypes and doses. If tendencies were observed with the increase in the dose for certain characteristics such as the narrowing of the 3rd leaf, the stem branching and the prostrate nature of the plant, for other characteristics such as the shape and the colour of the fruit we have observed effects at specific doses or within a given dose range. The doses that caused the most interesting variation are mainly those ranging from 200 to 600Gy. It would be appropriate to experiment with the production of more M1 seeds by multiplying the number of doses of irradiation within this range.Gamma ray irradiation has created genetic diversity linked to qualitative traits, which is a source of value for this fruit vegetable. These results will undoubtedly contribute to the selection of okra varieties in order to better meet consumer needs. It should however be noted that all the phenotypic modifications which appeared in M1 are not necessarily due to mutations. The continuation of observations in M2 remains a fundamental step for the characterization of all the plants obtained. Nevertheless, a selection for different interesting parameters can be expected, on the condition of a satisfactory variability in M2.

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