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**RESEARCH ARTICLE**

# VARIABILITY OF SOME AGRONOMIC PARAMETERS OF FIRST GENERATION OF MAIZE (*ZEA MAYS* L.) OBTAINED BY IRRADIATING SEEDS TO DIFFERENT DOSES OF GAMMA RADIATION

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**ABSTRACT**

Maize (*Zea mays* L.) is a cereal that has grown in all agricultural regions of the world. It is a cereal grown in a wide variety of growing conditions ranging from tropical to temperate climates. However, climate change, which is causing more intense drought followed by severe soil degradation, will lead to a reduction in the development and production of maize plants in northern Côte d'Ivoire. The aim of this study was to develop high-performance cultivars adapted to environmental conditions within a short period of time; Gamma radiation-induced mutation techniques were initiated. Three different gamma radiation doses (100; 200; 300 Grays) were applied to maize seeds of the variety EV8728. These seeds had grown and the technique of controlled self-fertilization was carried out as soon as the first flowers appeared. Data collected were analyzed with Statistic software. The impact of gamma irradiation at different doses on the growth and yield of first generation maize mutants was evaluated. Analyses of variance showed that the different irradiation doses had a very highly significant effect ($p < 0.001$) on all parameters studied. Stem heights showed a reduction of 7.41 % in stem height for 100 and 200 Grays dose followed by a reduction of 25.6 % for 300 Grays dose compared to the control. The weight of spikes with and without the spathes decreases with increasing radiation dose. The highest values were given by plants from controls (120, 26± 8,93a) and the lowest values were obtained from plants that seeds were irradiated at the 300 Grays dose (66,29 ± 16,51c). All, the results obtained indicate a significant reduction in the morphological and production parameters studied, except for sensitivity or resistance to lodging which is not related to the different doses. This reduction is accentuated at 300 Grays compared to the other radiation doses and the control. This effect is more or less important depending on the dose applied and is reflected in the biomasses or dimensions of the organs. For example, the use of gamma radiation has led to a morphological and physiological change in maize plants grown with new capabilities. These data have shown that gamma radiation can, at any dose, create drought resistant crop mutant to improve maize production in areas affected by severe soil degradation.

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## INTRODUCTION

Maize (*Zea mays* L.) is an annual herbaceous tropical plant of Poaceae family. It is one of the most widely consumed and grown cereals in the world (Von Braun *et al.*, 2010). Maize is an important food resource in tropical and subtropical regions. Its world production is estimated at 982 million tons with main producers being United States, China and Brazil (Statista, 2019). In Africa, maize production is more than 70 million tons from an area of 34 million hectares (Harold and Tabo, 2015).

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In Côte d'Ivoire, maize is the second most widely grown cereal after rice. Its annual production was 663780 tons, with a total area of 327 800 ha in 2018 (Statista, 2019). Maize cultivation accompanies the development of the Ivorian food trade, thanks to its storage potential, its high productivity and its location throughout the Ivorian territory. In forest areas, maize cultivation has gradually taken a considerable place by exploiting topographically high areas to complement rice, cassava, yam and taro cultivation (Kouakou *et al.*, 2010). For a long time considered as a simple subsistence product, maize is today the subject of agricultural speculation which is intensifying in Côte d'Ivoire with economic stakes that have become increasingly important. However, maize cultivation faces many problems caused by climate change, which is leading to increasing drought and soil degradation.

The main problems in maize cultivation are the high sensitivity of cultivars to poor soils and low rainfall (Benmahammed *et al*., 2010). Indeed, maize is a very water-intensive plant (Gong *et al*., 2015), especially during the two weeks before and after the appearance of silk (Farahani *et al*., 2014). Improving maize production therefore requires better control of the sensitivity of cultivars to climatic conditions. Since induction of mutations now occupies a prominent place in plant breeding. The use of gamma radiation as a source of increasing variability and specific improvement is necessary to create new varieties that guarantee maximum production under low water regimes significant changes in phenotype. Many experiments have shown that several traits such as earliness, resistance to diseases and pests, lodging resistance, drought resistance can be more easily obtained by artificial mutations than by hybridization or other ordinary breeding methods (Abdul *et al*., 2010). Thus, the use of Gamma radiation-induced mutation techniques to create high-performance varieties are of definite interest to Côte d'Ivoire since it offers the advantage of obtaining high-performance cultivars adapted to increasingly unfavorable environmental conditions in a short period of time. The general objective of this study is the development of high production and drought resistant maize varieties was initiated. This study was conducted to assess the effect of the irradiation dose to EV8728 maize seed on the growth and productivity parameters of plants grown from irradiated seed.

## MATERIALS AND METHODS

**Study site:** The work of the present study was carried out on the experimental site of Jean Lorougnon Guédé University of Daloa (Centre-Western Côte d'Ivoire). Daloa is located between 6°53'38 north latitude and 6°27'0 west longitudes and is part of Haut Sassandra region. The climate is tropical. The average temperature is 27.5 °C with an annual rainfall of between 1000 and 1500 mm. The soil at the study site is predominantly ferralitic (Soro, 2015). The work was carried out in the field.

**Plant material:** The study focused on the first generation of maize (*Zea mays*) obtained by seed irradiated the variety EV8728. EV8728 variety has a cycle from 105 to 110, yellow grains with a toothed texture and the average yield per hectare is from 3 to 5 t. The variety is tolerant to stripping and root lodging (Akanvou, 2006). The variety was supplied by the National Centre for Agronomic Research (CNRA) in Korhogo, Côte d'Ivoire.

**Seed preparation:** Dried maize seeds of variety EV8728 were irradiated at Genetic and Plant Breeding Laboratory of International Agency Energy Atomic (IAEA) in Seibersdorf, Austrilia. 1kg of seeds was divided into 3 lots. The different batches were irradiated respectively with gamma rays of 100, 200, and 300 Grays and sent for sowing to Daloa. Doses of 100, 200 and 300 Grays were chosen after radio sensitivity tests on corn seeds. The witness is represented by the uneradicated seeds.

**Preparation of the experimental device:** An experimental plot of 342m$^2$ (18 x 19 m) was used for the design of the experimental set-up. This plot was cleaned up and chicken droppings were buried in the ground. A fully randomized Fisher block device with two replicates was set up. Each rehearsal constitutes a block, with 152 m$^2$ per block. The distance between the two blocks is 2 m. Each block consists of 4 sub-blocks spaced 1 m apart. Each sub plot of 32m$^2$ has 11 lines spaced 0.8 m apart. Each line contains 11 bundles 0.4 m apart.

**Sowing and maintenance phase:** The maize seeds were sown manually to a depth of 3 cm with 2 kernels per bundles according to the experimental set-up. After 2 weeks sowing, 5 g of urea were applied around each bundles. The second cover fertilizer NPK (15,15,15) was applied the same time after urea application. Manual weeding of the plot was carried out at the time of seed emergence and again three weeks later. The plot is equipped with a drip irrigation system that has allowed the daily watering of the plot.

**Obtaining the $M_1$ generation plants by self-fertilization:** Sixty-seven (67) days after sowing, the male and female flowers were covered with craft paper envelopes as soon as they appeared. The cobs have been wrapped to avoid pollution by foreign pollen. For the panicle of the same foot, when it reaches maturity, pollen is collected in the envelope and transferred to the female flowers.

**Data Collection:** Growth parameters were measured at 67 days after sowing. The caulogenic parameters (concerning the stem) selected are:

- The Plant Height (HP) which is the distance from the base of the stem to the point of cob insertion;
- The Insertion Height (HIE) of cob which is the distance from the collar to the insertion point of the cob on the stem;
- The Collar Diameter is the circumference of the base plant at a height of 1 to 2 cm from the ground;
- The Verse Sensitivity Index (VSI) is calculated following this formula:
- VSI = HIE/ HP(11). When the results tend towards 1 then the plant is resistant to lodging.

**The phylogenetic parameters (leaf formation) measured are:**

- The Number of leaves obtained by counting from germination to the appearance of spikelet's;
- Leaf length (LngLf) is taken from the longest leaf observed on the plant;
- The Sheet Width (WS) is the measurement of the median of the sheet;
- The Leaf Area (LfA) is calculed as: LfA= LngLf x WS x 0.75(Lichtenthaler, 1987).

Data of production were collected at harvest, 153 days after sowing. The selected parameters are:

- The Number of cobs was obtained by counting each corn stalk;
- The Filling of Cobs has been established according to a scale from 1 to 3 corresponding to filling levels: 1: Fully filled cobs; 2: Medium filled cobs; 3: Lightly filled cobs;
- The weight of cobs with spathes and without spathes was determined using a scale.

**Statistical analysis:** The data of different vegetative parameters were performed using the Statistica7.1 software. The data obtained were subjected to analysis of variance and

Tukey's test was used to means comparison. Tukey's and variance's tests were used for the calculation and classification of the averages, respectively.

## RESULTS

**Effect of irradiation doses on caulogenic parameters:** The caulogenic parameters of mutated maize plants from irradiated EV8728seed were influenced by different irradiation doses (Table 1). Analyses of variance showed that the different irradiation doses had a very highly significant effect ($p < 0.001$) on all caulogenic parameters of different stalks of corn. The different results indicate that the stem height, the diameter of the collar and the insertion height of the cob decrease with increasing irradiation dose. Stem heights showed a 7.41% reduction in stem height for the 100 and 200 Grays dose followed by a 25.6% reduction for 300 Grays dose compared to the control. The classification of treatments made it possible to distinguish two different groups at 95% confidence level. The first group is composed of the control, 100 and 200 Grays dose while the second group is composed of 300 Grays dose.

For collar diameter, statistical analysis revealed also a significant reduction of 6.32% and 23.49% for the 100 and 300 Grays doses, respectively, compared to the control and a slight increase of 1.45% for the 200 Grays dose. The comparison of the averages identified two different groups identical to those of stem height parameter. For the insertion height of the cob, a significant reduction under the influence of different doses was observed. This reduction is 12.58%; 19.23% and 30.73% for the 100; 200 and 300 Grays doses, respectively, compared to the control. Comparison of averages identified 2 groups also. The control, 200 and 300 Grays doses make up Group 1, while the 300 Grays doses make up Group 2.

**Impact of irradiation dose on phylogenetic parameters:** The results on the effect of gamma irradiation dose on phylogenetic parameters of mutated maize plants from irradiated seed are reported in Table 2. The irradiation dose has a very highly significant effect ($p < 0.001$) on all phylogenetic parameters. The number of leaves, leaf length, leaf width and leaf area decreases as the irradiation dose increases. Statistical analysis reveals a significant reduction in the different parameters studied for plants from seeds irradiated with 100, 200 and 300 Grays doses compared to the control. Comparison of means of different phylogenetic parameters identified two different treatment groups, the first group consisting of control, the 100 and 200 Grays dose and the 300 Grays dose which constitutes the second group. These results show that gamma radiation induces a significant reduction in phylogenetic parameters. The various parameters decrease effectively as the irradiation dose increases.

**Influence of gamma radiation dose on production parameters:** The results on the effect of different gamma irradiation doses on the production parameters of mutated maize are shown in Table 3. The number of cobs is represented by the appearance of individual cobs on the corn stalk. The highest values are obtained from plants derived from seeds irradiated at the 300 Grays dose ($1.7 \pm 0.24$) and the lowest from control plants ($1.16 \pm 0.12$). The number of cobs harvested increases with increasing radiation doses. Comparison of averages identified two treatment groups. Group 1 consists of control, 100 and 200 Grays dose and Group 2 consists of 300 Grays dose. The weight ofcobs with and without spathes decreases with increasing radiation dose. The highest values are given by plants from non irradiated seeds and the lowest values are obtained from plants from seeds irradiated at the 300 Grays dose. Comparison of the averages for these 2 parameters revealed 3 treatment groups. Group 1 consists of the control, 100 and 200 Grays dose were group 2 and Group 3 consists of 300 Grays dose. With regard to the filling ofcobs , the results show an increase with the increasing radiation dose. The statistical analysis identified three groups. The first one is the control; group 2 is composed of the 100 and 200 Grays radiation and finally group 3 is composed of the 300 Grays dose. At the level of the lodging sensitivity index, the results indicate a very small variation of $0.42 \pm 0.01$ to $0.44 \pm 0.21$. Statistical analysis indicates that no significant difference ($p>0.05$) was observed between plants from irradiated seed and the control. The analysis of results showed that various production parameters decrease with the increase of the irradiation dose with the exception of the number of cobs and the filling of cobs which increases with the dose. No significant effects of different irradiation doses were observed on the lodging resistance index. On the other hand, the different irradiation doses have a very highly significant effect ($p < 0.001$) on all production parameters studied except for the lodging resistance index ($p > 0.05$).

## DISCUSSION

The use of gamma radiation at doses of 100, 200 and 300 Grays on EV8728 maize seeds has induced an influence in the different parameters studied. However, these parameters behave differently depending on the applied radiation dose. At the 100 and 200 Grays doses, the reduction in parameters is significantly lower compared to the 300 Grays dose where the reduction is very high. This result indicates that the irradiation doses used inhibit plant growth. In general, increased irradiation doses have detrimental effects on plant growth, this is consistent with the (Saiful *et al*., 2015) work, who showed that higher doses of gamma radiation are detrimental to the growth of young grape plants. The work on curcuminoid plants (*Curcuma longa)* showed that the maximum height of the plant is obtained at lower doses of gamma irradiation (Ilyas and Naz, 2014).

This size is reduced as doses become higher and higher. The results of this study showed that high doses of irradiation induced a significant reduction in the size of the maize plants grown from the irradiated seeds. However, this author states that low-dose gamma radiation (50 and 100 Grays) induces an increase in plant growth and an increase in the number of *Canscoradecurrens* nodes (Yadav, 2016). Similar results were reported by (Abdul *et al*., 2010) on *Lepidium sativum.* According to these authors, the number of leaves, the height and the number of nodes of this plant decrease with increasing gamma irradiation doses. This work has shown that this drop can go up to the death of the plant for a dose of 300 Grays. This could be explained by the fact that a high dose and a long duration of irradiation would lead to the destruction of plant cells (Jan *et al*., 2012). Indeed, the high dose of irradiation increased inhibition and arrest of the cell cycle and caused genome damage (Preuss *et al*., 2003). For these authors, ionizing radiation caused biological effects, mainly changes in DNA and RNA. They observed changes such as chain breaks or hydrogen bonds. These observed changes resulted in the blocking of DNA replication.

**Table 1. Influence of gamma radiation on stem parameters in mutated maize plants**

| Treatment (Gy) | Stem height (cm) | Insertion height of the ears (cm) | Diameter at the collar (mm) |
|---|---|---|---|
| 0.0 | 172,4 ± 7,4a | 72,89 ± 2,63a | 16,45 ± 0,63a |
| 100 | 159,61 ± 3,78a | 63,8 ± 3,46a | 15.41 ± 0.58ab |
| 200 | 153,36 ± 3,24a | 58,87 ± 2,55a | 16,69 ± 0,45a |
| 300 | 128,27 ± 29,94b | 50,49 ± 5,57b | 12,6 ± 3,96b |
| P | < 0,001 | < 0,001 | < 0,001 |

In a column, values followed by the same letter are not significantly different at 5%. P: Probability

**Table 2: Influence of gamma radiation on phylogenetics traits in mutated maize plants**

| Treatment (Gray) | Number of Sheet | Length of Leaves (cm) | Width of the Sheets (cm) | Leaf area (cm2) |
|---|---|---|---|---|
| 0.0 | 17,75 ± 0,41a | 82,27 ± 2,7a | 8,24 ± 0,21a | 509,31 ± 28,95a |
| 100 | 16,69 ± 0,4a | 79,82 ± 2,24 [a] | 7,8± 0,18a | 469,61 ± 18,22a |
| 200 | 16,88 ± 0,48a | 78,22 ± 1,84 [a] | 7,76 ± 0,15a | 455,87 ± 17,15 [a] |
| 300 | 15,79 ± 0,6 [b] | 69,67 ± 3,75b | 6,98 ± 0,21b | 365,52 ± 30,02b |
| P | < 0,001 | < 0,001 | < 0,001 | < 0,001 |

Values followed by the same letter are not significantly different at 5% in a column. P: Probalité

**Table 3. Influence of gamma radiation on mutated maize production parameters**

| Treatment (Gray) | Number herringbone | Spike weight With spathe (g) | Spike weight Without spathe (g) | Filling of spikes | Index of Resistance to pouring |
|---|---|---|---|---|---|
| 0.0 | 1,16 ± 0,12a | 138,25 ± 7,11a | 120,26 ± 8,93a | 1,27 ± 0,07c | 0 ,42 ± 0,01a |
| 100 | 1,17 ± 0,06a | 112,35 ± 13,35b | 96,13 ± 10,27b | 1,53 ± 0,13b | 0,39 ± 0,02a |
| 200 | 1,34 ± 0a | 101,65 ± 8,45b | 82,64 ± 6,3b | 1,95 ±0,2b | 0,38 ± 0,01a |
| 300 | 1,7 ± 0,24b | 80,13 ± 15,13c | 66,29 ± 16,51c | 2,71 ± 0,14a | 0,44 ± 0,21a |
| P | < 0,001 | < 0,001 | < 0,001 | <0,001 | 0,728 |

In a column, values followed by the same letter are not significantly different at 5 %. P: Probability

When there is no distribution system for the type of binding created, protein synthesis is stopped and oxidation destroys the lipoprotein structure of the membrane. All these disturbances lead to inhibition of cell growth and even death (Sanaa, 2013). This shows that low doses of radiation are able to induce auxin synthesis and high doses destroy auxin activity (Liu *et al.*, 2008).The set of changes caused by irradiation may be the inhibition of seed germination (Rozman, 2014). And, very low doses are effective in controlling germination (Bonhomme, 2003). The doses used depend on the variety, nature and origin of the products as well as environmental conditions. This study also revealed that the different irradiation doses had a very significant effect on the leaf area of maize plants.

This result shows that low doses of gamma radiation irradiation of seeds have stimulating effects on the leaf surface (Lebon, 2006). High doses have a depressive effect on the evolution of the photosynthetic surface. Indeed, according to (Lebon, 2006), irradiation of corn seeds causes stress in the plant. This water stress causes a significant reduction in the photosynthetic surface area. Lack of water causes a decrease in evapotranspiration through leaf senescence and a reduction in leaf area. In addition, the influence of irradiation on maize production parameters showed that the different irradiation doses had a highly significant reducing effect on mutated maize production parameters. This result is in line with those of (Jan *et al.*, 2012), who showed that 100, 200 and 300 Grays doses induce a significant reduction in production parameters. And, those from the works who showed that the number of okra fruits per plant and the length of the fruits following irradiation with gamma radiation had decreased with a high irradiation dose of 250 Grays (Dubey *et al.*, 2007). For these authors, a dose of 250 Grays would inhibit flower formation, hence the absence of fruit formation. This shows that for good growth and production of plants from irradiated seed, a reasonable or low dose should be used. High doses could affect the synthesis of auxins that are responsible for nucleic acid synthesis, leading to hormone synthesis in plant cells, causing plant development (Jan *et al.*, 2012). This work has thus shown that susceptibility or resistance to lodging is not related to different doses. This parameter is related to different factors such as leaf index, root system, length and diameter of basal internodes and semi density (Yonggui *et al.*, 2015). The lodging has an impact on plant yield and depends on the cultivar (Shi *et al.*, 2016). These results show that doses 100, 200 and 300 Grays have a very significant effect on the weight of the different ears. This indicates that these doses have an inhibitory effect on hormone synthesis, which would prevent the organs of the plant from developing properly. These results are consistent with work that has reported that the most common effect observed in plants after irradiation is growth retardation or cessation (Jones *et al.*, 2004)). This effect is more or less important depending on the dose applied and is reflected in the biomasses or dimensions of the organs. These same results were also obtained on soybeans where they observed a decrease in weight compared to the control at very high doses of ionizing radiation (300 grays) (Jan *et al.*, 2012). The results of this study showed a 66.29% decrease in spike weights without spathes at the 300 Grays dose. For example, dry mass reductions were observed after irradiation of 10 Grays in *Psicum sativum,* or 20 Grays in *Holcuslanatus*. Research has shown that a dose of 50 Grays would increase the yield of the rice seed and the height of the different plants (Maity *et al.*, 2005). While that others works showed that a very high dose (300 Grays) made the rice plants sterile and reduced their height by half (Cheema *et al.*, 2003). According to the results and observations made in the field, the use of ionizing radiation would allow a morphological (decrease) and physiological change on plants. This result shows that gamma radiation is capable of creating culture mutants at any dose (Feng *et al.*, 2019).

These purified and homogeneous mutants could improve maize production in the savannah areas of northern Côte d'Ivoire, which are affected by severe climate-induced soil degradation.

Conclusion

The study of the influence of the three doses (100, 200 and 300 Grays) of gamma ionizing radiation on the morphological and yield parameters of the first generation of maize of the mutated EV8728 variety revealed a significant difference between the control plants and those from seeds irradiated with gamma radiation. It was found that gamma radiation has an effect on the parameters studied. Ionizing radiation induced the appearance of morpho-physiological changes depending on the radiation dose. Thus, the increase in gamma radiation dose reduced the morphological parameters of the different EV8728 maize plants mutated and also had an impact on maize production. The 300 Grays rate generally reduces plant growth compared to the 100 and 200 Grays rates, therefore there is a lack of dry matter production. This reduction in growth results in a decrease in all growth parameters compared to the control. The doses applied negatively influenced the majority of the productivity parameters analyzed. The most affected trait was plant productivity determined mainly by ear weight and ear filling. These results indicate a relationship between gamma radiation dose and production quality. However, the induced mutation technique applied to EV8728 offers better prospects for achieving our goals.

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**Glossary of Abbreviations**

CNRA: National Centre for Agronomic Research
IAEA: International Agency Energy Atomic

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