

Agro-morphological performances of M₃ generation of irradiated maize developing against the Fall Armyworm in Central African Republic

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Comment [a.s1]: In the manuscript, the author did not test the resistance of maize to fall armyworm. It is better if the title is changed slightly so as not to confuse the reader.

Abstract:

Productivity of ~~Maize-maize~~ in Central African Republic (CAR) is further reduced by the invasion of Fall Armyworm. The use of developing genetic resistance in maize against the pest remains under-explored. This present study was conducted to determine the effects of radiation on maize growth and development. The test with different doses of irradiation (C controls, 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy) was performed on three varieties (CMS85 01, CMS-20 19 and CMS87 04) of maize and on the local ecotype of CAR.

The effect of varieties and differences doses on the growth parameters (plant survival, number of leaves, length of leaves, larger of leaves, P plant height diameter of collet;) and yield parameters (number of cobs, number of rows per cob, numbers of grain per cob) was performed using ANOVA 2 with R software version 3.1.3. The Shapiro-Wilk test to normalize the data and differences tests using a significance level of 0.05. The Principal Analysis of Component was used to analyse the relationships between the growth parameters and yield parameters of in different treatments.

Few number of leaves are observed from local ecotypes than the all varieties, following by CMS 2019 with exception from dose 400 Gy. The two others varieties respectfully CMS 8501 et CMS 8704 have sensibly equal number of leaves. Statistically, the significant difference (P-value= 4.363e-05) was observed between different varieties according the different doses of irradiation (P-value= 6.665e-16).

The height from different varieties according the different doses are equal, exception the dose 400Gy of CMS 2019. Statistically, the difference observed is not significative. But, the height of 400 Gy from the variety CMS 2019 is less important than the rest of doses from others varieties.

The most less quantity of grains (228) was obtained from the CMS 2019 variety at 400Gy and the most important quantity (2040) from the same variety at 300 Gy. produced the most less number of rows per cob. The CMS 8704 variety at 100 Gy produced the most important number of rows per cob.

Statistically, there is the significant difference according to the maize varieties (P-value= 0.0014874) and the different doses of irradiation (P-value= 0.865586).

Keywords: gamma radiation, growth parameters, reproduce parameters, maize.

1. Introduction

Zeamays is one of the world's most important crop plants, boasting a multibillion dollar annual revenue ([1], [2]).

The increment of maize production in the world is very important due to the fact that this cereal is used for human and animal consumption as well as to produce energy, making it increasingly important to develop new technologies to increase its production.

In addition to its agronomic importance, maize has been a keystone model organism for basic research for nearly a century [3].

In Central African Republic (CAR), i) Maize comprises a lucrative market that has great potential for growth, ii) Maize production is an engine for economic growth and generates substantial income per

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unit area and per person, iii) Maize creates new income opportunities by value adding activities especially for small farmers([4], [5]).

Productivity of Maize in CAR is further reduced by the invasion of fall armyworm (FAW), a pest that has been creating excessive crop losses since 2016([6], [7]). The FAW, *Spodoptera frugiperda*, is a pest that is native to the tropical and subtropical regions of the Americas. It can feed on more than 80 plant species including Maize([8], [9]).

But, as a model organism, maize is the subject of far-ranging biological investigations such as plant domestication, genome evolution, developmental physiology, epigenetics, pest resistance, heterosis, quantitative inheritance, and comparative genomics([10], [11], [12]). Increasing productivity is a constant challenge for the maize production chain, and higher levels can be achieved when some management practices are changed.

While a variety of biological, chemical, agronomic and transgenic techniques are being tested and used for crop protection against FAW, breeding for genetic resistance has lagged behind.

However, since the advent of transgenic crops modified with Bt genes, breeding for resistance to insect pests has not been explored in any depth. Recently, resistance to the brown plant hopper in rice was demonstrated in mutant plants with a mutation in a single cytochrome P450 gene([13], [14]). Thus, the likelihood for genetic resistance in maize and groundnut to insect pests exists but remains under-explored.

The utilization of nuclear techniques in the area of agriculture, defense, and power generation has increased over the last few decades([15], [16], [17]). Radiation technology is widely used to produce changes in product characteristics leading to the development of new products. Gamma irradiation was found to increase plant productivity([18], [19], [20]).

Considering the effects of radiation on plants, the present study was conducted to determine the effects of radiation on maize growth and development.

2. Materials and Methods

2.1. Vegetable material

The test of performance from irradiated seeds was performed on three varieties (CMS85 01, CMS-20 19 and CMS87 04) of maize and on the local ecotype of CAR. The details of the characteristics of maize varieties used in this study are in table 1.

Table 1: Characteristics of maize varieties used in the study

Variety	Genetic nature (N Gq)	Origin & Year of release	Date of introduction or registration in CAR	Cycle (days)	Height of Plants(cm)	100 seed weight (g)	Colour of seed	Seed Texture	Potential Yield (t/ha)	Organoleptic characteristics and suitability for processing
CMS85 01	Composite	IRAD 1985)	1988	105-110	180-220	24.5	White	Cornea - dentate	5 - 8	Susceptibility to lodging, drought and stem borers
CMS-20 19	Composite	IRAD 1990)	1994	85-90	140-170	22.5-8	White		4 - 5	Drought and disease tolerance, Striga ensitivity
CMS87 04	Composite	IRAD 1987)	1988	105-110	190-240	24.5	Yellow	Cornea	7 - 8	Sensitivity to lodging, good resistance to drought, very high sugar content
Local ecotype										

2.2. Experimental test

The experiment was carried out at the Regional Polyvalent Research Center (CRPR) of Boukoko (05°04' South latitude, 018°49' East longitude and 499 m altitude) in Region of Lobaye. It is a tropical type of climate marked by two main seasons (the rainy season from March to April and October to November and the dry season from J November/December to Feb [16]).

Comment [a.s2]: This research was carried out during the dry season?? Or during the rainy season?? What year?

The experimental design adopted was the complete block plan with three repetitions (R1, R2 and R3) and six treatments (Controls, 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy) [18]. There are four plots of 40 m long by 14 m wide for an area of 560 m² each. The repetitions were separated by 3 m in distance and 2 m of border at the ends. Sowing was carried out at spacings of 1 m on the line and 1.5 m between two lines at the rate of two seeds per pocket. A first application of inorganic manure (NPK) was carried out one day after the first weeding and the second application of inorganic manure (Urea) at the appearance of the male inflorescence.

Comment [a.s3]: What dose to use ?

2.2.1 Growth measuring parameters

a) The plant survival

The plant survival (%), was measured at two weeks and four weeks after sowing after sowing. The Survival percentage for each replication is calculated according to the following formula [19]:

Survival percentage = $\frac{\text{Number}}{\text{Number of seeds planted}} \times 100$

Comment [a.s4]: What amount ? Number of live plants ?

b) Number of leaves

The number of leaves per plant is obtained by counting every two weeks while taking into account the leaves located at the base.

c) Length of leaves

The length of leaves is measured at centimeters from node to terminal part of the same leaf every two weeks. During maturity it is the leaf of the main ear that we measured. We used a tape measure for the measurements.

d) Larger of leaves

Leaf width Leaf widths are measured in centimeters on the middle part of the leaves every two weeks. At maturity, it is the leaf of the main ear that we measured.

Comment [a.s5]: Width of leaves ???

e) Plant height

Leaf width Leaf widths are measured in centimeters on the middle part of the leaves every two weeks. At maturity, it is the leaf of the main ear that we measured.

f) Diameter of collar

The collar diameter is measured in centimeters at the base of the stem every two weeks.

2.2.2 Yield measuring parameters

g) Number of cobs per plant

The number of cobs per plant is counted on the plants at field level during harvest.

h) Number of rows per cob

The Number of rows per cob was estimated by counting the grains in the cob taking into account the rows without grains. The rows are counted by hand after the ears have dried.

i) Number of grains per cob

Number of grains per cob was estimated by counting the number of grains in maturity.

a) Average seedling height = $\frac{\text{Sum of seedling in mm}}{\text{Number of plants measured}} \times 100$

The measured parameters were in the four plots: (i) emergence rate (%), calculated two weeks after emergence; (ii) plant height (mm), measured with a tape measure at two weeks and four weeks after sowing; (iii) plant survival (%) at two weeks and four weeks after sowing. The percentage of

germination or emergence of seedlings (a), survival (b) and variation in seedling height (c), for each replication is calculated according to the following formula [19]:

- b) Germination percentage = Number of germinated seeds / Number of seeds planted x 100
- c) Survival percentage = Number of surviving seedlings / Number of seeds planted x 100
- d) Average seedling height = Sum of seedling height in mm / Number of plants measured x 100

2.3 Data analysis

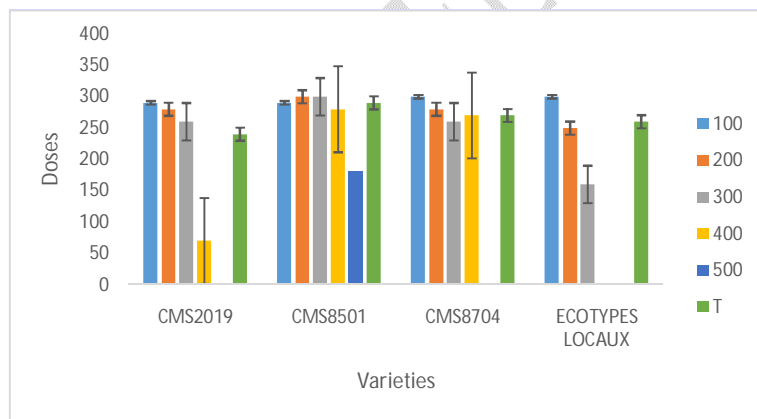
The effect of varieties and differences doses (0 Gy, 100 Gy; 200 Gy; 300 Gy; 400 Gy; 500 Gy) on the growth parameters (plant survival, number of leaves, length of leaves, larger of leaves, Plant height diameter of collet,) and yield parameters (number of cobs, number of rows per cob, numbers of grain par cob) was performed using ANOVA 2 with R software version 3.1.3. The Shapiro-Wilk test to normalize the data and differences tests using a significance level of 0.05.

The Principal Component Analysis was used to analyse the relationships between the growth parameters and yield parameters of in different treatments.

3. Results

3.1 Plant survival test

Data from the differences doses (0 Gy, 100 Gy; 200 Gy; 300 Gy; 400 Gy; 500 Gy) of plant survival from three varieties (CMS85 01, CMS-20 19 and CMS87 04) of maize and on the local ecotype of CAR are reported in figure 1. With the higher doses (500 Gy), plants did not survive for CMS 2019, CMS 8704 and local ecotypes. All plants were survived for all doses with CMS 8501. But with local ecotypes, 400 Gy and 500 Gy, plants did not survive. Statistically, high significant difference (P-value= 0.001484) was observed between different varieties according the different doses of irradiation (P-value= 0.00009358).



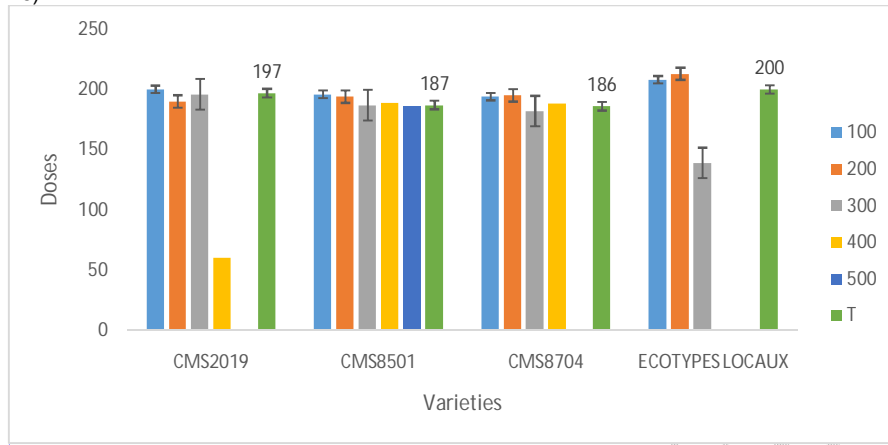
Comment [a.s6]: This diagram is difficult to understand. Redraw the diagram, making the Y-axis as the percentage of living plants.

Figure 1 : Plant survival from different varieties according the different doses

3.2 Number of leaves

Few number of leaves are observed from local ecotypes than the all varieties, following by CMS 2019 with exception from dose 400 Gy (fig.2). The two others varieties respectively CMS 8501 et CMS 8704 have sensibly equal number of leaves. Statistically, the significant difference (P-value= 4.363e-05) was

observed between different varieties according the different doses of irradiation (P-value= 6.665e-16).

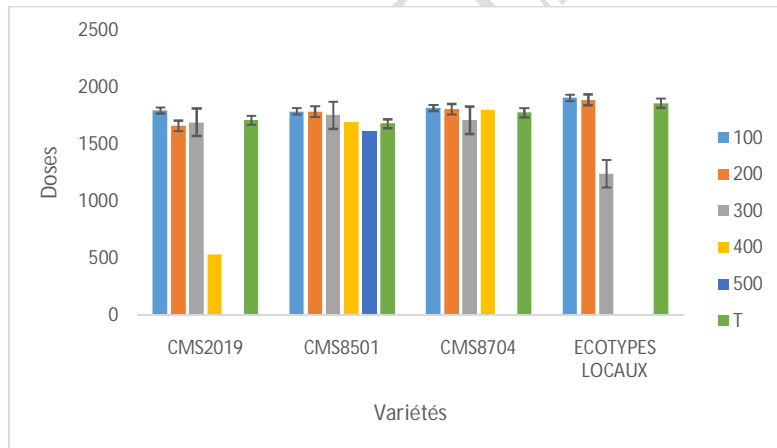


Comment [a.s7]: This diagram is difficult to understand. Redraw the diagram, making the Y-axis as the number of leaves.

Figure 2: Number of leaves from different varieties according the different doses

3.3 Length of leaves

The local ecotypes, exception 300Gy presented leaves with important length than the CMS 8501. The doses 100 and 200 Gy increased most length from local ecotypes. But 400 Gy, decreased length of leaves from the variety CMS 2019 (fig3.). Statistically, the significant difference (P-value= 0.0003981) was observed between different varieties according the different doses of irradiation (P-value=2.045e-08).

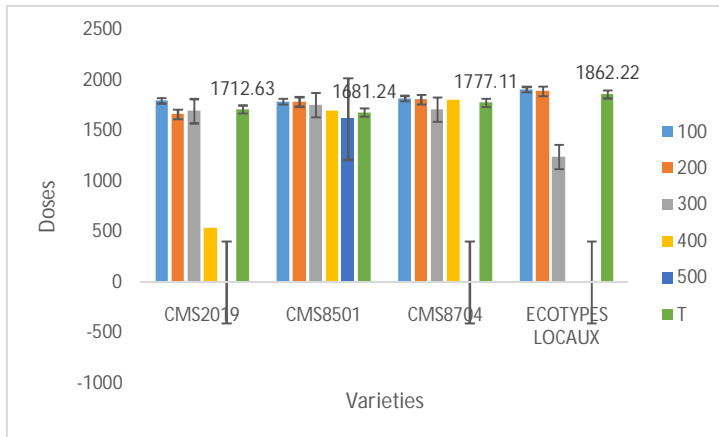


Comment [a.s8]: This diagram is difficult to understand. Redraw the diagram, making the Y-axis as the length of leaves.

Figure 3 : Length of leaves from different varieties according the different doses

3.4 Larger of leaves

The local ecotypes, exception the dose of 300Gy presented important larger of leaves the CMS 8501. The doses 100 and 200 Gy increased most larger leaves from local ecotypes. But 400 Gy, decreased larger of leaves from the variety CMS 2019 (fig.4). Statistically, the significant difference (P-value= 0.0072) was observed between different varieties according the different doses of irradiation (P-value= 0.00002508349).

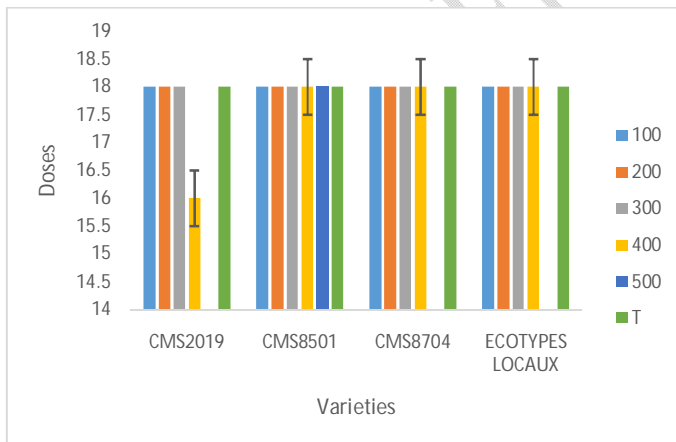


Comment [a.s9]: This diagram is difficult to understand. Redraw the diagram, making the Y-axis as the width of leaves.

Figure 4 : Variation of the larger of leaves from different varieties according to the different doses

3.5 Plant height

The height from different varieties according to the different doses are equal, exception the dose 400Gy of CMS 2019. Statistically, the difference observe is not significant. But, the height of 400 Gy from the variety CMS 2019 is less important than the rest of doses from others varieties in culture (fig.5).



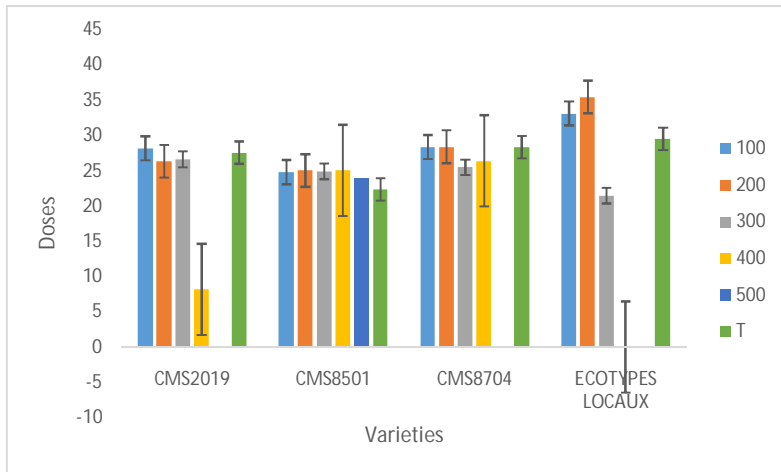
Comment [a.s10]: This diagram is difficult to understand. Redraw the diagram, making the Y-axis as the plant height.

Figure 5 : Variation of Plant height from different varieties according to the different doses

3.6 Diameter at collar

The variance analysis showed that there is significant difference (P-value= 0,06934) between the varieties (fig.5).

Comment [a.s11]: Describe which variety and at what mutation dose has the largest diameter?

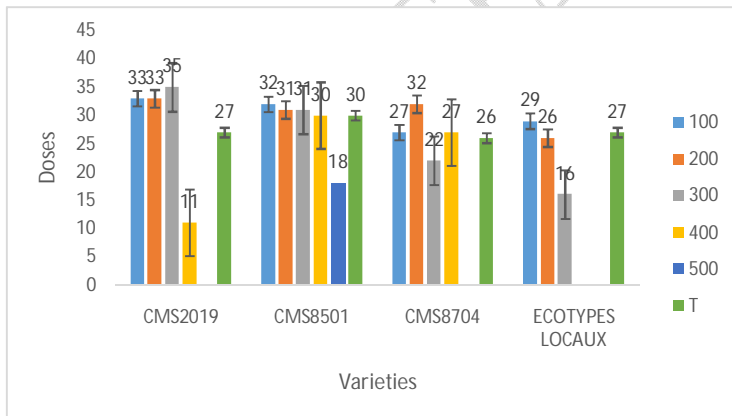


Comment [a.s12]: This diagram is difficult to understand. Redraw the diagram, making the Y-axis as the diameter at colar.

Figure 6 : Variation of diameter of colar from different doses per maize variety

3.7 Number of cob

The variety CMS 2019 increases important number of cob than the varieties CMS 8501, CMS 8704 and local ecotypes (Fig 7). The dose of 300 Gy from CMS 2019 increases the most number of cob than the dose 400 Gy. Statistically, the significant difference (P -value = 0.004154) was observed between different varieties according to the different doses of irradiation (P -value = 0.042729).



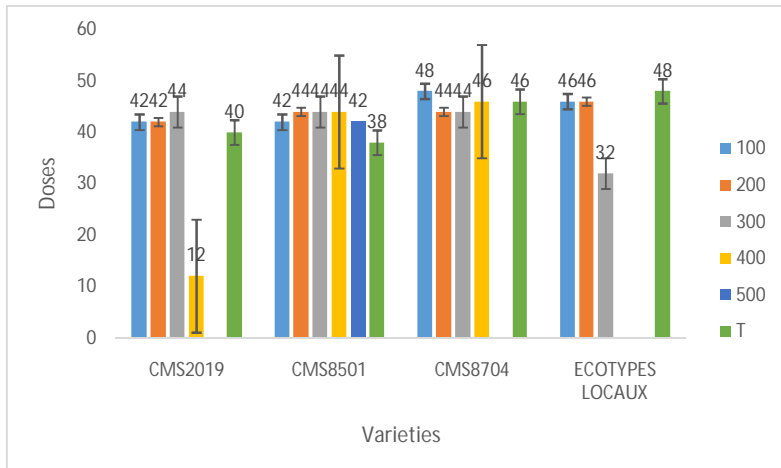
Comment [a.s13]: This diagram is difficult to understand. Redraw the diagram, making the Y-axis as the number of cob.

Figure 7: Variation of number of cob from different doses per maize variety

3.8 Number of rows per cob

The statistical analysis showed an important variability according to number of rows per cob from different doses per maize variety (figure 8). The CMS 2019 variety at 400 Gy produced the most less number of rows per cob and 500 Gy is lethal. The CMS 8704 variety at 100 Gy produced the most important number of rows per cob compared to the control. However 300 Gy and 400 Gy are lethal for local ecotype.

Statistically, there is the significant difference according to the maize varieties (P -value = 0.03585) and the different doses of irradiation (P -value = 0.01676).



Comment [a.s14]: This diagram is difficult to understand. Redraw the diagram, making the Y-axis as the number of rows per cob.

Figure 8 : Variation of rows per cob from different doses per maize variety

3.9 Number of grains per cob

The figure 9 showed the number of grains per cob from different doses per maize variety. The most less quantity of grains (228) was obtained from the CMS 2019 variety at 400 Gy and the most important quantity (2040) from the same variety at 300 Gy. The CMS 8704 variety at 100 Gy produced the most important number of rows per cob. Statistically, there is the significant difference according to the maize varieties (P -value = 0.0014874) and the different doses of irradiation (P -value = 0.865586).

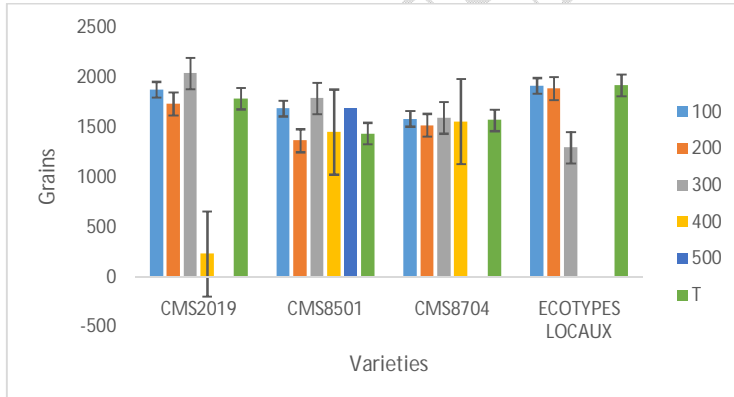


Figure 9 : Variation of grains per cob from different doses per maize variety

3.10 Principal Analysis of Component for the growth parameters

The Principal Component Analysis performed on the different growth parameters showed correlations between these different parameters (fig.10). The two main axes explained 99.17% global variability. The first axis contributed to 97.24 % with parameters: diameter of colar, length of leaves, number of leaves. The second axis contributed to 1.93 % with the plant survival (%) parameter. The different parameters according to two factorial axes are strongly correlated. But the variability concerns only the plant survival (%) parameter.

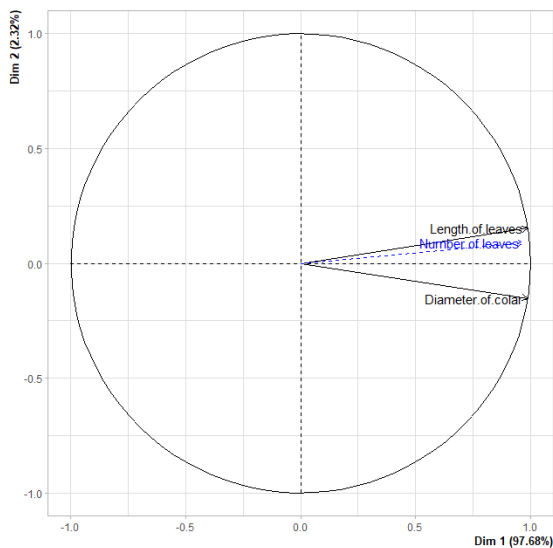


Figure 10 : Principal Analysis of Component for the growth parameters

3.11 Principal Analysis of Component for the yield parameters

The Principal Component Analysis performed on the different yield parameters showed correlations between these different parameters (fig.11). The two main axes explained 95.3% global variability. The first axe contributed at 79.42% with parameters: number of cob, number of rows per cob, Number of grains per cob. The second axe contributed at 15,88 %. The different parameters according to two factorial axes are strongly correlated.

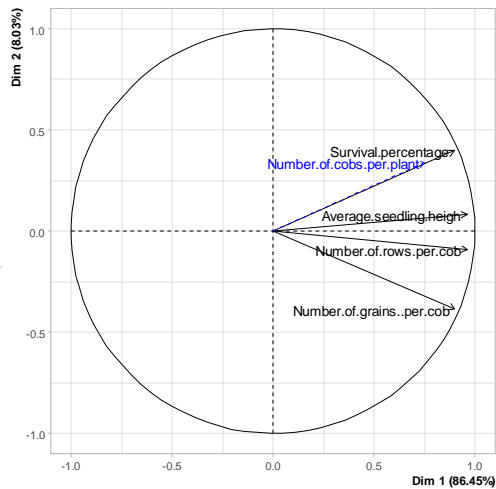


Figure 11 : Principal Analysis of Component for the yield parameters

4. Discussion

Comment [a.s15]: I notice from the data you got, CMS8501 is the only variety that can grow and produce seeds. It would be better if you explain why this could happen?

The seed germination test after gamma irradiation (0Gy, 100 Gy; 200 Gy; 300 Gy; 400 Gy; 500 Gy) revealed that the maximum germination percentage was observed in control seedlings. As illustrated in Fig.1, the final germination percentage decreased with increasing gamma ray doses by (300 Gy, 400 Gy, 500 Gy) according to the different varieties. The maximum decrease, of the germination percentage was observed from the CMS 2019 variety at 400 Gy.

In several studies, lethal and stimulatory effects of gamma irradiation on germination percentage, emergence, and survival of seedlings of different plant species have been reported.

The effects of gamma radiation are investigated by studying plant germination, growth and development, and biochemical characteristics of maize. Maize dry seeds are exposed to a gamma source at doses ranging from 0.1 to 1 kGy, moreover, plants derived from seeds exposed at higher doses (≤ 0.5 kGy) did not survive more than 10 days [23].

Also it has reported that germination of seeds can be influenced in both positive and negative directions by gamma radiation exposure as a result of mutation inductions depending on cellular abnormalities or stimulatory modifications triggered by radiation doses [24].

The results from [25] showed that the seedling length increased in lower doses (0.0005 to 0.1 kGy) of gamma irradiation and reduced on and beyond the 0.2 kGy dose for both the genotypes. Similarly seedling vigor index I reduced on and beyond 0.2 kGy of gamma irradiation dose. The germination per cent and alpha amylase activity was significantly more in lower doses (0.0025 to 0.2 kGy) of gamma irradiation and reduced on 0.3 kGy or beyond for both maize genotypes [25].

Plant radiosensitivity is dependent upon several factors, some related to plant characteristics (e.g. species/cultivar/variety, plant age, physiology, tissue architecture, morphology and genome organization) and some related to radiation features (type of radiation, dose rate and time of exposure) ([26], [27], [28]).

According to the parameters length of leaves, our result showed that the local ecotypes, exception 300Gy presented leaves with important length than the CMS 8501. The doses 100 Gy and 200 Gy increased most length from local ecotypes. But 400 Gy, decreased length of leaves from the variety CMS 2019 (fig.3.).

The results from ([29], [30], [31], [32], [33]) showed that X-ray significantly affected plant growth (plant height and plant dry matter) and some growth indices (crop growth rate, relative growth rate, absolute growth rate, and leaf area ratio), yield and yield components (number of seeds, weight of seed and grain yield) of maize as well as nutritional value of the crop and leaf growth.

According to [34], the maximum radical length was recorded in the control samples, while the radical length of samples exposed to 0.1, 0.2, 0.3, 0.5 and 0.7 kGy decreased by 9, 31, 41, 47, and 56%, respectively. The maximum reduction of radical length, by 71%, was observed at 1 kGy. Results show that gamma treatment with doses higher than 0.1 kGy significantly inhibited the length of the radicular system of plants derived from irradiated seeds.

Our result showed that the height from different varieties according to the different doses are equal, exception the dose 400Gy of CMS 2019, but with high dose (400 Gy), the height is less important (fig.5).

The results from ([35], [36]) showed that the gamma rays (≥ 0.2 kGy) imposed a significant impact on the shoot length. The highest shoot length was observed in the unirradiated plants. Following exposure to gamma rays, shoot lengths decreased by 11, 40, 48, 53, and 60% at 0.1, 0.2, 0.3, 0.5, and 0.7 kGy, respectively. The maximum decrease of shoot length, by 63%, was observed at 1 kGy [37].

The statistical analysis showed an important variability according to number of rows per cob from different doses per maize variety (figure 8). The CMS 2019 variety at 400Gy produced the most less number of rows per cob and 500 Gy is lethal. The CMS 8704 variety at 100 Gy produced the most important number of rows per cob compared to the control. However 300Gy and 400 Gy are lethal for local ecotype.

The figure 9 showed the number of grains per cob from different doses per maize variety. The most less quantity of grains (228) was obtained from the CMS 2019 variety at 400Gy and the most important quantity (2040) from the same variety at 300 Gy. The CMS 8704 variety at 100 Gy produced the most important number of rows per cob.

The results from [38] showed that the biological yield, grain weight in cob and 100 grain weights respond positively to the lower doses (0.1kGy) of gamma irradiation and improvement in biological

yield by 35,2% at 0.1kGy as compared to the control (00kGy). However, maximum reduction (33%) was recorded at 0.5 KGy. Similarly, grain yield was improved by 8,3% at 0.1kGy as compared to the control, but reduced the most by 56,9% at 0,5 kGy compared to the respective control.

The irradiation effect of the maize sowing seeds stimulates an increase in seed yield. An analysis of the yield structure showed that an increase in maize yield resulted from an increase in row number per ear, [39].

5. Conclusion

The effects of gamma radiation are investigated by studying plant germination, growth and development characteristics of maize (CMS85 01, CMS-20 19 and CMS87 04) ~~of maize~~ and on the local ecotype). The gamma radiation with high dose (400Gy) had a depressive effect on growing and yield parameters in maize plants. The degree of sensitivity of the plants depends on the dose of irradiation and the nature of the plants.

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Comment [a.s16]: Are you sure the 400 Gy dose has the greatest effect? What about the 500 Gy dose that causes some varieties to be unable to grow based on your data?

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