ASSESSMENT OF THE SUSCEPTIBILITY TO FUSARIUM EAR ROT OF CORN PLANTS GROWN FROM SEEDS OF VARIETY EV 8728 IRRADIATED WITH GAMMA RAYS

ABSTRACT

This study aimed at determining the effects of various doses of gamma rays on the susceptibility to Fusarium ear rot and the yield parameters of corn seeds of variety EV 8728. The experiment was carried out at the experimental station of the University Jean Lorougnon Guédé on a randomized complete block plot with 3 repetitions. An isolate of local *Fusarium* sp. local served as the source of contamination. The inoculation technique used was the injection of spore suspensions into the silk channel. Corn kernels were irradiated with 200 and 300 Grays gamma rays, while the unirradiated variety EV 8728 was used as a control. The results showed that the control corn ears were more susceptible to Fusarium ear rot. Their severity (1.59 %), intensity (1.51 %) and incidence (91.67 %) were higher than those of the ears resulting from irradiated seeds, which were respectively 0.52 %, 0.49 % and 83.33 % for 200 Grays doses, and 0.25 %, 0.22 % and 80.56 % for 300 Grays doses. Statistical analysis of these values showed a significant difference ($p < 0.01$) for susceptibility parameters. As for yield and yield parameters, they were strongly influenced by irradiation. Control plants had higher yields than irradiated plants ($p < 0.01$), meaning that the 200 and 300 Grays doses inhibited productivity. However, some corn plants were identified whose ears showed resistance to Fusarium ear rot at the 200 Grays dose, and whose yield was statically similar to that of the control. The corn kernels grown from these plants could be the subject of in-depth studies so as to provide starting genitors for a breeding program for corn varieties resistant to Fusarium ear rot.

Keywords : Fusarium rot, isolate, irradiation, corn, yield, susceptibility.

INTRODUCTION

Corn (*Zea mays*) is one of the most important cereals in people's diets. Its energy reserves represent 80 to 84 % of fresh grain total weight, and it is considered an ideal food for provitamin A fortification (Deffan et al., 2015). Vitamin A is essential at all stages of life. It is involved in many bodily functions, including vision. Corn plays an essential role in the food self-sufficiency of populations in Côte d'Ivoire, particularly in areas with average and erratic rainfall. It is grown in all agroecological zones, from the south in the Sudanian climate to the north in the Sudano-Sahelian climate, although its preferred zone is between isohyets 600 and 900 millimeters (northern zone) (Akoudjin et al., 2016). However, despite strategies to steadily increase yield, corn cultivation in Côte d'Ivoire still has a low level of productivity, with an average yield of less than 1.5 t/ha (Akanvou et al., 2009). In fact, corn cultivation remains dependent on rainfall, which is variable both in time and space, and above all characterized by drought pockets. Added to this are diseases, which considerably reduce yield. Among the latter is a particular case, Fusarium ear rot, which affects yields but also the health quality of the harvest through the presence of toxins in kernels (Schurch, 2016). This endemic disease is caused by a complex of species of phytopathogenic fungi, the "fusarium complex", with a broad host spectrum (Miedaner, 1996). In recent years, the disease incidence has considerably increased worldwide. Today, this disease is one of the most worrying scourges for farmers. Infected ears exhibit a shrunken appearance and/or yield small, whitish, deformed and practically empty grains (Leonard & Bushnell, 2003). Plots attacked by Fusarium show losses in yield and crop quality, resulting in considerable economic losses for farmers. In addition to these yield losses, Fusarium produces mycotoxins (Schachermayer & Fried, 2000**)**, the best known of which is deoxynivalenol (DON) or vomitoxin. DON is found in harvested products such as corn flour and livestock feed. DON production in kernels represents a real danger to human and animal health (Uéno et al., 1983).

Many cultivated corn species are susceptible to Fusarium rot. Now, infected corn residues and contaminated kernels are an ideal inoculum reservoir (Dill-Macky & Jones, 2000). To date, fungicide treatments have not yielded suitable results (Bailey et al., 2004), and the safest way to combat Fusarium rot is a genetic solution incorporating resistant cultivars (Clements and *al*., 2003). For sustainable corn production in Côte d'Ivoire, the selection of varieties resistant to both drought and Fusarium ear rot could be an important and effective means of ensuring food security.

This study is part of a vast project initiated by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA), in collaboration with the University Jean Lorougnon Guédé in Daloa, which is entitled: "(Basic Mutation Breeding Technique) Daloa, Côte d'Ivoire TC Project IVC 5039 Improving Crops Subject to Severe Soil and Climate Degradation trough Induced Mutant Adapted to these Area''. The main objective of this study is to assess the response of corn plants grown from seeds irradiated with gamma rays to the local isolate of fusarium ear rot of corn.

MATERIALS AND METHODS

Experiment site

The study was conducted in Daloa, precisely at the experimental site of the University Jean Lorougnon Guédé. This university is located in the Tazibouo neighborhood in the northeast of the city, precisely between longitude West 6°26'17" and latitude North 6°54'32". The locality has a humid tropical climate, with average annual rainfall varying between 1 400 and 1 800. The region has two rainy seasons a year, from April to mid-July, with drought pockets, and from September to November.

Materials

The plant material used in this study consisted of corn plants grown from irradiated seeds of variety EV8728 (Table 1). Two irradiation doses, 200 and 300 Grays (Figure 1), were tested and compared with the control (variety EV872). The seeds of corn variety EV 8728 came from the germoplasm of the National Center for Agronomic Research of Korhogo (Ivory coast) and were irradiated at the IAEA Genetic and Plant Breeding Laboratory in Seibersdorf, Austria. The corn variety EV8728 is a high-yielding variety that has yellow grains that are highly appreciated by the population.

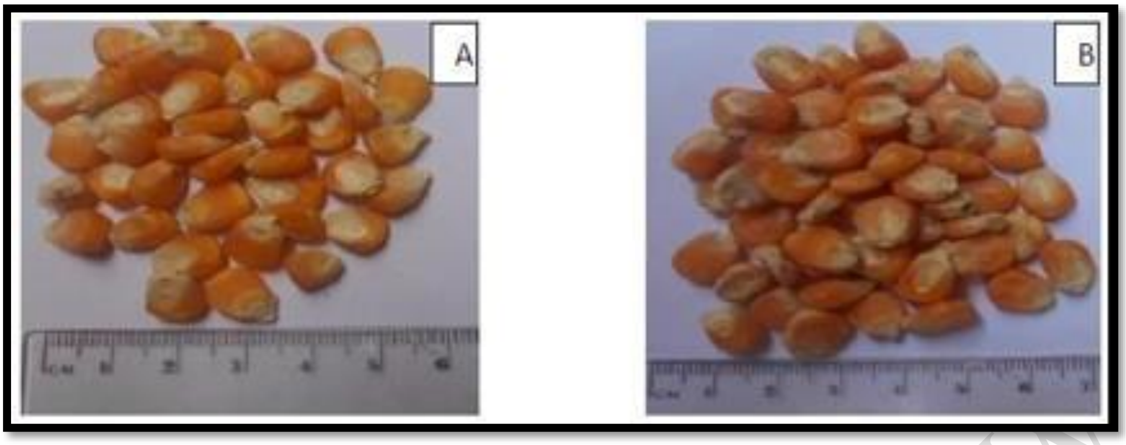

Figure 1. Irradiated corn seeds with 200 Grays (A) and 300 Grays (B).

Table I. Characteristics of corn variety EV8728

Variety	Characteristics			
	Maturity cycle	**Kernel color and texture**	**Specificity**	**Average yield (t ha^{-1})**
EV8728	Intermediary (105-110 days)	Yellow, dent	Tolerant to stripe and root lodging	3-5

The fungal material used in this study came from a natural isolate of local *Fusarium* sp. This was a corn ear containing fusarium-damaged kernels, purchased at the Daloa town market. The isolate of *Fusarium* sp. was obtained on PDA culture medium (Piperno et al., 2009). Isolated fungi were identified and characterized on the basis of macroscopic observations and binocular loupe observations for highlighting the presence of fruiting bodies, including mycelium. The mycelium produced was suspended in sterile distilled water and shaken so as to release the zoospores. The resulting suspensions were filtered in order to separate the conidia from the mycelium. The concentration of spores in the inoculum was one Petri dish per 10 ml of sterile distilled water.

Setting up the experiment

The study took place over 4 months (May to August 2019) in a 20 m^2 wooden shelter. Thus, the set-up began with the plot clean-up. This was followed by the installation of a wooden shelter covered with netting so as to protect the plants from pests. Finally, elementary plots were delimited. Crop buckets of 30 cm height and 27 cm in diameter, each with a capacity of 10 l, were filled with soil taken from the 0-30 cm horizon of a plot that had been left fallow for 5 years. The resulting growing medium was treated with a 75 g l^{-1} fungicide (mancozeb). The experimental design chosen was a randomized complete block with 3 repetitions (Figure 2). Each block contained 2 elementary plots 2 m long by 1 m wide. The distance between two elementary plots in the same block was 1 m. The distance between 2 blocks was 0.6 m. Each of the elementary plots comprised 9 crop buckets. Within each bucket, 3 corn kernels were sown, keeping distances of 0.70 m in length and 0.40 m in width between buckets. Plot maintenance consisted in regularly watering the corn plants for keeping them in excellent water conditions. Manual weeding was also carried out throughout the cultivation period so as to eliminate interspecific competition. Fifteen (15) days after germination (plant emergence), the plants were pruned in order to leave the most vigorous ones. Fertilizer was applied at a rate of 3 g of N15 P15 K15 per corn plant 15 days after sowing (DAS), then 35 days after sowing, urea was incorporated into the soil at a rate of 100 kg per ha.

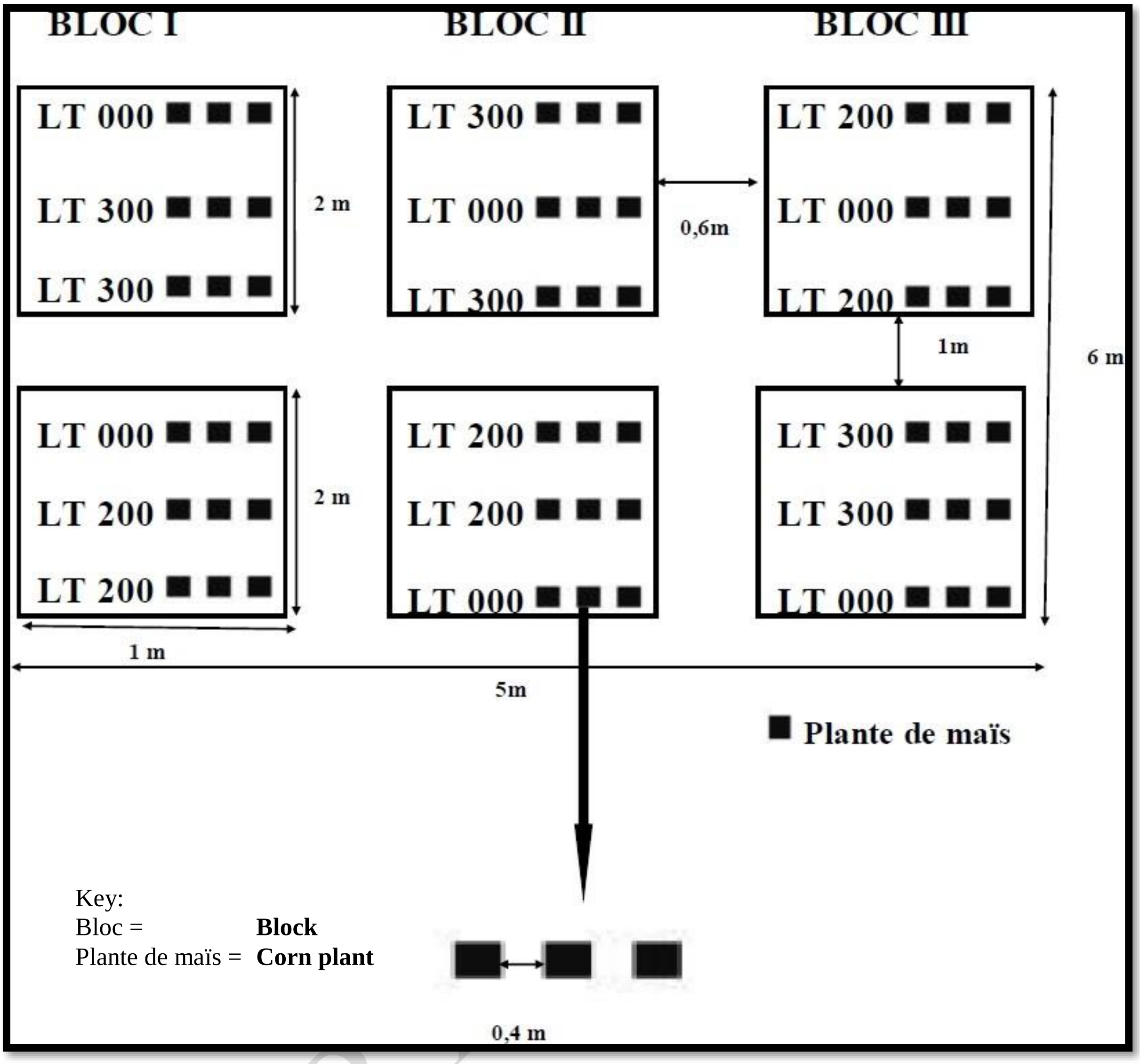

Figure 2. Experimental design

LT000: Row comprising the control treatment (unirradiated corn), **LT200**: Row comprising the 200-Grays dose irradiated corn treatment, **LT300**: Row comprising the 300-Grays dose irradiated corn treatment.

Inoculation of corn plants grown from variety EV8728 seeds

Plants were inoculated at the flowering and silk production stage of corn. It is at this precise stage of development that differences in susceptibility are greatest among grasses (Schurch, 2016). For inoculation, the method of injecting spore suspension into the silk canal was used as described by Schurch in 2016. To this end, 1 ml of spore suspension was injected on the ear tip using a syringe, 5 to 7 days after silk emergence (female flowering). This inoculation was carried out in the evening, starting as from 6 pm. Plants with inoculated ears were marked with a blue plastic tag (Figure 3).

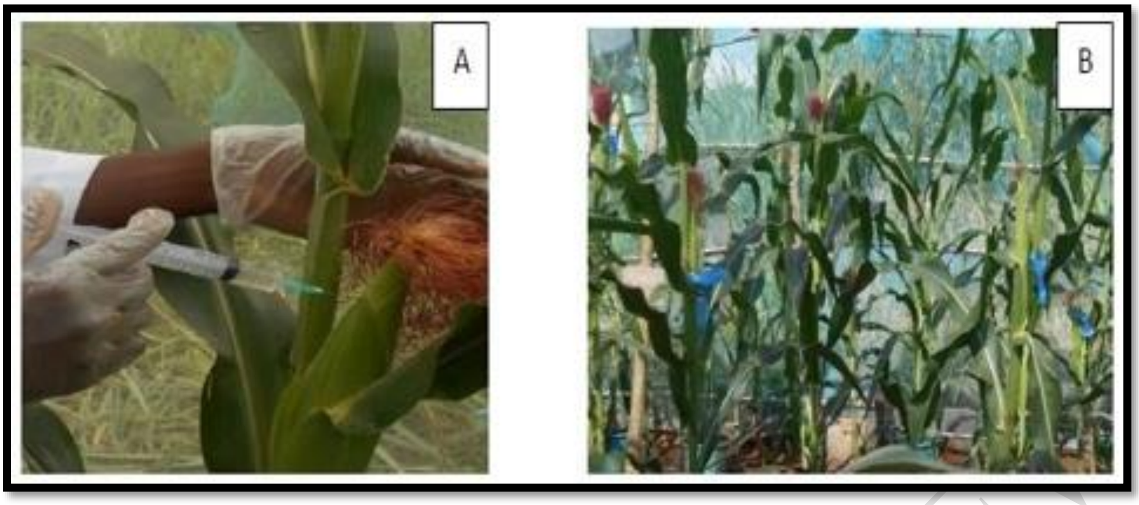

Figure 3. Inoculation of ears by injection (A) and labelling of inoculated ears of plants (B).

Observations and measurements

Assessment of *Fusarium* sp. isolate

Assessment was carried out in the laboratory and focused on the color of the fungus, the appearance of the mycelium and its growth. Mycelial growth was measured at 2, 3, 4 and 5 days after incubation. To this end, 2 perpendicular lines passing through the center of the explant were drawn on the Petri dish. The lines were used to measure mycelial colony diameters. Color and appearance were assessed after each daily observation.

Assessment of the susceptibility of corn plants

The disease was assessed in the field. For this purpose, the ears were freed from their spathes and the parameters of severity, incidence and intensity of Fusarium blight were calculated. For this purpose, the Paulina (2015) and Lemmens (2012) rating scales were used. Paulina's scale assigns a percentage based on the surface area of the affected ear, while Lemmens' scale is based on the number of fusarium-damaged kernels. Thus, 500 kernels were taken as the unit, then one diseased grain represented 0.2 % of disease severity (Table 2).

Table 2. Ear rot assessment scale according to Lemmens (2012) with a slight modification

Scale (%)	**No. of infected kernels**	**Scale** (%)	**No. of infected kernels**
0	0.0	**15**	75
0.2	1	**25**	125
0.4	2	**35**	175
0.6	3	**50**	250
1	5	**75**	375
3	15	**90**	450
5	25	**100**	500
10	50		

Based on the rating scales, Fusarium ear rot severity (S) was expressed as a percentage of infected ear tissue (infected kernels) in relation to the total number of kernels in the ear, using the formula:

$$S\ (\%) = \frac{\text{Infected tissue's surface area}}{\text{Tissue's total surface area}}\ X\ 100 \qquad \textbf{(1)}$$

Disease incidence was calculated from the ratio between the number of infected ears and the total number of inoculated ears assessed per treatment. It was estimated as a percentage using the following equation:

$$I\ (\%) = \frac{\text{Number of infected ears}}{\text{Total number of inoculated ears assessed}}\ X\ 100 \qquad \textbf{(2)}$$

Disease intensity (DINT) was calculated as the multiplicative product of the percentage of infected ears and the severity of infection on the ear. This intensity was calculated according to the formula:

$$(\%)\ \text{DINT} = \frac{(\text{Disease incidence X disease severity on the ear})}{100} \qquad \textbf{(3)}$$

Assessment of production parameters

At harvest, the assessment of production parameters included ear mass, kernel mass per ear, ear length and yield. Ear mass per plant was obtained after harvesting the spathe-free ears, which were then dried to a constant mass. The mass of the kernels from each ear was then obtained after shelling and weighed under the same conditions as the ears. Yield ($t.ha^{-1}$) was calculated using the formula below :

Yield (t. ha - 1) = average mass of infected or inoculated ears x surface area cultivated **(4)**

Data analysis

Data collected in the field were entered and processed using EXCEL 2013 spreadsheet and STATISTICA 7.1 software. They were then subjected to analysis of variance (ANOVA) at α = 0.05 threshold. Multiple comparison tests (Tukey HSD) were used to classify significantly different means.

RESULTS AND DISCUSSION

Results

Isolation and characterization of *Fusarium* sp. local strain

Inoculation of Fusarium-damaged corn kernels purchased on the local market on PDA medium made it possible to identify *Fusarium* sp. local strain (Figure 4). Indeed, examination of the macroscopic appearance of the fungus showed a white, cottony texture with rapid growth (Figure 4 A). Microscopic observation made it possible to see partitioned mycelia with crescent-shaped conidia (Figure 4 B). These conidia were mostly segmented into 6 lodges (septae) (Figure 4 C). This local strain was isolated and kept cool in order to study its pathogenesis on irradiated and unirradiated corn variety EV8728.

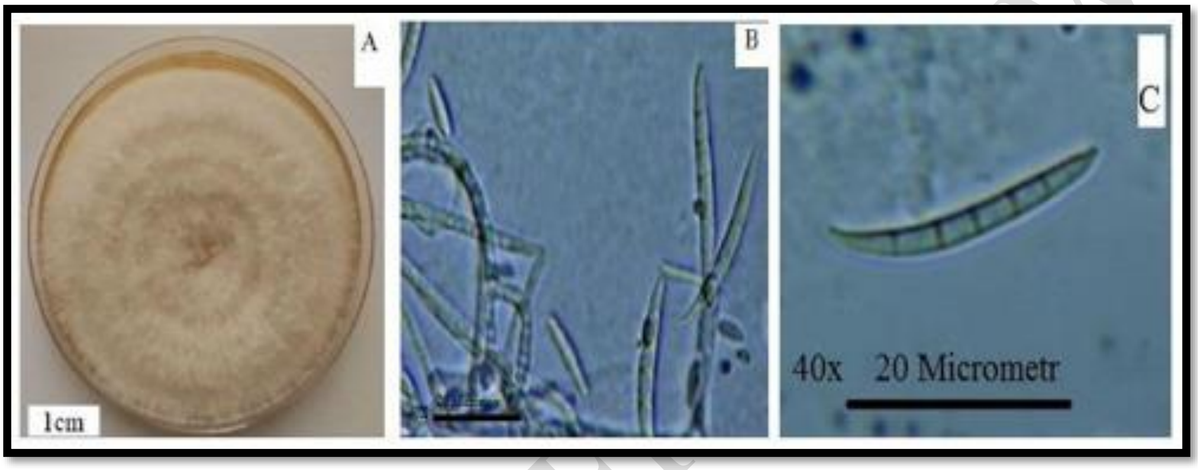

Figure 4. Macroscopic (A) and microscopic observation of local strain of *Fusarium* sp. (B, C).

Pathogenicity of *Fusarium* sp. local strain

Characteristic symptoms of the presence of *Fusarium* sp. were observed at corn ear harvest. Thus, on contaminated ears, the fungus appeared in the form of fine cotton (mycelium), white then wine-pink in color, located at the top of the ear at the start of the attack (Figure 5). Susceptible plants then showed stuck silks and spathes, rotten cobs and dented kernels. In some cases, all the kernels in a single corn crown were colonized.

Figure 5. Ears infected with *Fusarium* sp.

Assessment of corn variety susceptibility

The susceptibility of irradiated or unirradiated corn variety EV8728 to gamma rays was assessed using 3 parameters including disease severity, disease intensity and disease incidence (Table 3).

Fungal inoculation made possible ear colonization by *Fusarium* sp., whatever the irradiation dose of the seeds planted. However, colonization severity varied with irradiation. The highest values were found in plants grown from the control (1.59 %) and the lowest ones in plants grown from seeds irradiated at 200 and 300 Grays (Figure 6). Comparison of the means made it possible to identify 2 different groups, the first being the control and the second, the 200 and 300 Grays doses. Statistical analysis showed a significant difference ($p < 0.001$) between these 2 groups.

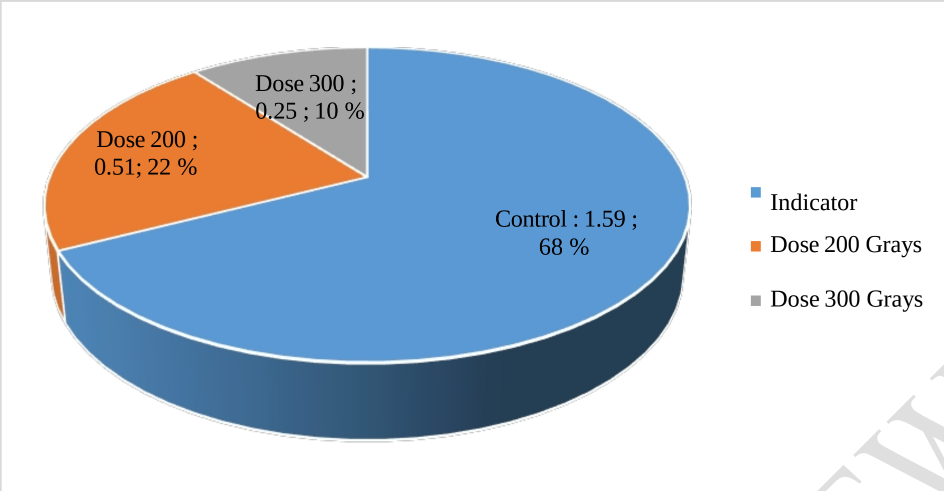

Figure 6. Disease severity in the different treatments of corn EV8728.

As for Fusarium rot incidence, it was higher in the control than in the plants grown from irradiated seeds, ranging from 91.67 to 80.56 %. The highest values were found in plants grown from the control (91.67 %), and the lowest ones in plants grown from seeds irradiated with 200 and 300 Grays (83.33 and 80.56 %). However, statistical analysis showed that there was no significant difference between these values (Table 3).
Disease intensity being the product of disease severity and incidence, it was reduced in plants of the seeds irradiated with 200 and 300 Grays compared with the control. In relation to the control, this reduction rate was 21.7 % and 25.41 % for 200 and 300 Grays doses, respectively. Tukey's HSD test made it possible to identify 2 distinct groups and an intermediate group ; the first one being the control, the second one being 300 Grays doses, and the intermediate one being 200 Grays doses. The results of the statistical analysis showed a significant difference ($p < 0.01$) between the 2 groups (control and 300 Grays) in relation to disease intensity (Table 3).

Table 3. Effect of *Fusarium* sp. on the different corn treatments

Treatment	Disease severity (%)	Disease incidence (%)	Disease intensity (%)
Control	1.59 ± 0.53^{a}	91.67 ± 14.43^{a}	1.51 ± 0.68^{a}
200 Grays	0.52 ± 0.23^{b}	83.33 ± 14.43^{a}	0.49 ± 0.26^{ab}
300 Grays	0.25 ± 0.11^{b}	80.56 ± 17.34^{a}	0.22 ± 0.12^{b}
p	< 0.001	NS	< 0.01
F	12.88	0.42	7.75

The values followed by the same letter are not significantly different within a column (5 % HSD Tukey test) ; NS : not significant

Yield assessment

Four parameters - ear length, ear mass, kernel mass per ear and yield - were used to assess the effect of *Fusarium* sp. on yield after ear drying. Statistical analysis of the results showed a significant effect ($p < 0.01$) for all the parameters studied, depending on irradiation doses (Table 4). Indeed, assessment of average ear mass revealed that inoculation of corn plants with *Fusarium* sp. induced a variation. This variation ranged from 135.63 to 64.16 g. The highest masses were found in the control plants (135.63 g) and the lowest ones in the plants treated with 300 Grays dose (64.16 g). As for the average kernel mass of inoculated ears, a variation was observed ($p < 0.001$). Tukey's HSD test at 5 % threshold indicated the presence of 3 groups, namely the control and 200 and 300 Grays irradiation doses. Thus, kernel mass in the control group was 73.53 g on average, and was higher than the one in 200 and 300 Grays doses. In terms of average ear length, statistical analysis revealed a highly significant difference ($p < 0.001$) between treatments. The highest values were observed in the ears of plants grown from the control (17.57 cm) and 300 Grays irradiation doses (11.80 cm). Following the difference in ear length, the presence of 2 groups was identified using Tukey's HSD Test at 5 % threshold.

Table 4. Effect of inoculation on corn ears

Treatment	Dry weight of the inoculated ear (g)	Dry weight of ear kernels (g)	Ear length
Control	135.64 ± 8.71^{a}	73.53 ± 4.59^{a}	17.57 ± 1.20^{a}
200 Grays	82.52 ± 2.59^{b}	49.04 ± 1.10^{a}	13.10 ± 2.23^{b}
300 Grays	64.16 ± 8.10^{c}	32.27 ± 3.7^{a}	11.80 ± 2.38^{a}
p	< 0.001	< 0.001	< 0.001
F	83.84	108.54	18.99

The values followed by the same letter are not significantly different within a column (5 % HSD Tukey test)

Yield

The results showed a variation from 2.36 to 1.11 t ha^{-1} (Figure 7). The highest values were held by the control (2.36 t ha^{-1}) and the lowest ones by 300 Grays irradiation doses (1.11 t ha^{-1}) (Table 5***)***. Comparison of the means revealed the presence of 3 groups consisting of the control, 200 Grays doses and 300 Grays doses. Statistical analysis showed a significant difference ($p < 0.001$) in yield between these 3 groups.

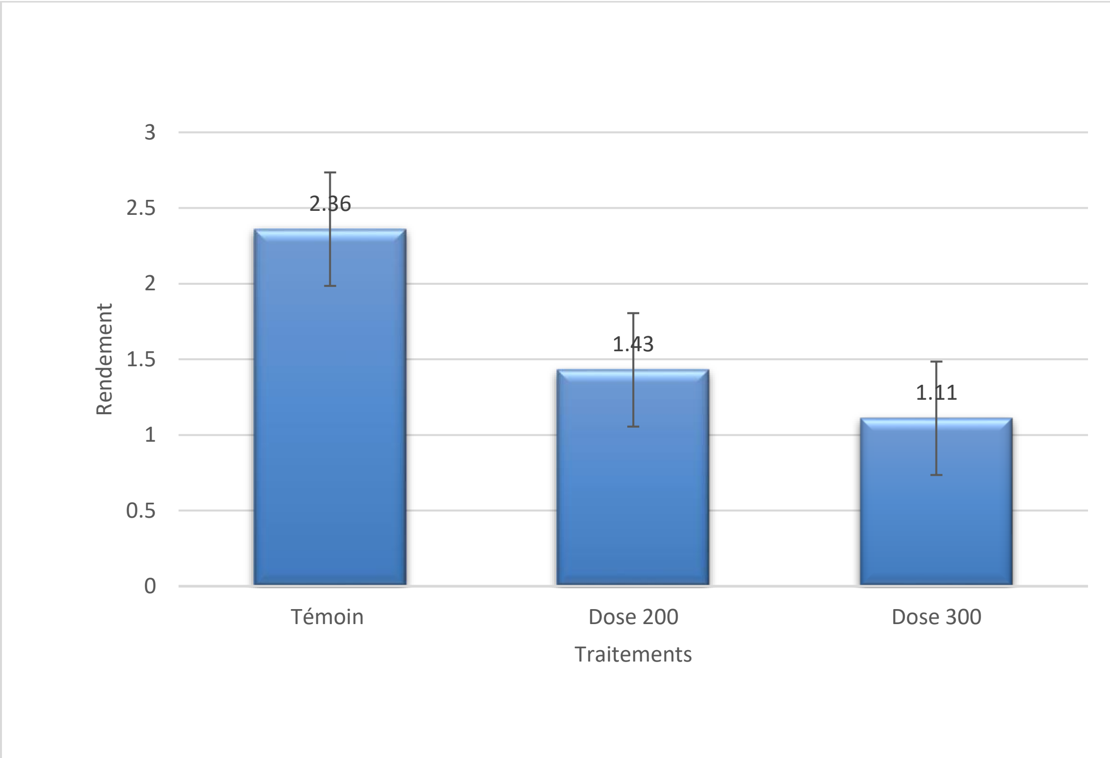

Figure 7: yield of different corn treatments

Table 5. Effect of inoculation on yield

Treatment	Yield (T.ha^{-1} of infected ear)
Control	2.36 ± 0.21^{a}
200 Grays	1.43 ± 0.16 ^{b}
300 Grays	1.11 ± 0.12^{c}
P	< 0.001
F	82.46

The values followed by the same letter are not significantly different within a column (5 % HSD Tukey test); NS : not significant

Discussion

This study, conducted as part of IVC 5035 Project, aimed at assessing the susceptibility of gamma-irradiated corn kernels to Fusarium rot. To this end, the inoculum of a local strain of

Fusarium sp. was assessed on corn plants of variety EV8728 irradiated or not with gamma rays. This isolate was obtained from a corn ear, containing rotten kernels, purchased at the market in the town of Daloa. The local inoculum thus obtained was injected into corn plants and made it possible to observe at harvest corn ears exhibiting typical Fusarium ear rot symptoms. The incidence of Fusarium rot in the crop shows inoculation success and *Fusarium* sp. virulence. Indeed, the precautions taken in setting up the experiment showed that the spread of the disease on all the plants grown from the different treatments (control 200 and 300 Grays) was due to their inoculation with the local strain of *Fusarium* sp. Now it was important to assess the susceptibility of corn plants to Fusarium rot and the result of this inoculation on corn yield and productivity.

According to Chupin et al. (1988), yield losses due to Fusarium ear rot can be estimated from counts of kernels and surfaces colonized by the fungus mycelium. In this study, this was achieved by assessing the severity, incidence and intensity of the disease on corn plants. We noticed that Fusarium ear rot was more severe on plants grown from unirradiated corn seed than on plants grown from irradiated corn. This result might suggest that, under the effect of gamma irradiation, variety EV8728 has developed resistance mechanisms to Fusarium rot. Indeed, according to Chenal et al. (2000), a living organism subjected to irradiation develops effective repair and detoxification mechanisms, enabling it to re-establish cellular equilibrium and survive stress. Moreover, the variation in disease intensity reveals that the level of attack differs according to the treatment. Indeed, a high disease intensity was observed in control plants, whereas in plants treated with 300 Grays the intensity of Fusarium rot was low. This suggests that the higher the irradiation dose, the greater the resistance of the irradiated organism to *Fusarium* sp. attack. Our findings are similar to those of Kurimoto et al. (2010), who have shown that the level of irradiation plays an important role in the plant's response to a stimulus.

However, yield parameters and kernel yield were not correlated with either the severity of Fusarium rot or its incidence in the crop. Indeed, corn ear mass, kernel mass per ear, ear length and yield per ear mass of variety EV8728 were significantly higher than those of irradiated plants. This shows that the incidence of Fusarium rot in the crop had no impact on yield. Two factors may explain the lack of correlation between visual quantification of symptoms and yield. These include the timing and duration of inoculation. As Fusarium ear rot is mainly seed-borne (Ky & Diomandé, 2017), it develops gradually as the plant grows and develops. In this context, the fungus has time to cause maximum crop damage. According to Pirgozliev et al. (2003), disease incidence in crops results in huge yield losses due to flower abortion, reductions in the number and mass of kernels, and changes in the quality of kernels. Now, in this study, inoculation took place at corn ear silk formation. The fungus had just enough time to colonize the ears before harvesting began, so the very short time between contamination and harvest was not enough to cause damage. The choice of inoculating plants at corn silk emergence stage had the effect of demonstrating the susceptibility to Fusarium rot of the different treatments applied to the variety. According to Prouillac (2006), artificial infections make it possible to overcome variability and identify physiological and genotypic differences.

As a result, it was gamma irradiation that induced an inhibition of productivity. This inhibition of growth led to a drop in yield parameters and yield per ear mass in irradiated corn plants. Already in a similar study on soybean populations subjected to irradiation doses of 200 and 400

Grays, Mongrain et al. (2000) revealed a significant reduction in most soybean agro-morphological parameters. As a result, Kurimoto et al. (2010) suggested the use of irradiation doses in the tens of Grays range for optimal growth in plant yield. However, the ideal irradiation dose must depend on the objectives being pursued. In the case of this study, a dose of 200 Grays might be suitable for reducing the susceptibility of variety EV8728 to Fusarium ear rot. In addition, some corn kernels irradiated at this dose produced plants with disease-resistant ears and good yields. The offspring of these plants can be used as progenitors in a Fusarium ear rot breeding program.

CONCLUSION

This study has shown that corn variety EV 8728 is susceptible to the local isolate of Fusarium sp. However, when irradiated with gamma rays (200 and 300 Grays), the severity and intensity of Fusarium ear rot was reduced. Disease intensity is a highly sensitive parameter, representing indicators of the degree of tolerance to irradiated doses. Increasing the irradiation dose reduces the susceptibility of corn variety EV 8728 to Fusarium ear rot. However, although unirradiated EV 8728 plants were the most susceptible to the disease, they exhibited the highest yield and yield parameters compared with plants grown from irradiated seeds. Thus, the different doses of gamma irradiation induced an inhibition of productivity. This study made it possible to show the resistance of some corn plants to Fusarium ear rot at 200 Grays dose. These individuals could serve as starting genitors for a Fusarium ear rot-resistant variety breeding program.

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