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Using Radiation-Induced Novel Genetic Diversity to Develop Pest-Resistant Maize in Central African Republic

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Abstract:

Maize is a major crop feeding 80% of the population of the Central African Republic (CAF). However, the country is not yet self-sufficient in its maize need and continues to rely partially on imports. Exacerbating this situation is the recent invasive spread of the fall armyworm attributed to change in weather patterns since 2016 which has been decimating the crop leading to drastic yield losses. Various control measures continue to be tested globally for the control of FAW, including chemical control, biological control using microbial organisms and predatory insects that attack FAW, use of genetically modified crops with Bt genes, and integrated pest management. However, the use of developing genetic resistance in maize against the pest remains under-explored. We focused research on initiating a mutation breeding effort in maize in CAF.

2000 seeds on four varieties (CMS 87 04, CMS 20 19, CMS 85 01, ECOTYPES LOCAUX) sending for irradiation to Vienna. 200 seeds are bulk to irradiation with different doses (100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy). The test of germination is carried out on 200 seeds four varieties. The treated seeds are sown at equal depths in a tray filled with soil/compost or can as well sow in natural condition on the field just to assure that the soil surface is flat, and the treated seeds are sown at equal depth containing the five treatments in rows of 200s seed each for one control and each treatment. Per assay three replicates are performed, one tray per replicate. Fourteen days after sowing, the seedling height and survival is measured to determine the Growth Reduction Value 50 or GR50.

All seeds from different doses (CMS-20 19, CMS87 04 and the Local ecotype) were germinated from different doses (100Gy, 200Gy and 300Gy) and did be presented the symptom of FAW. However, for the CMS85 01, all seeds from different doses (100Gy, 200Gy, 300Gy; 400Gy and 500Gy) were germinated as the control, but the major of plants are attacked by the FAW (Fall Armyworm).

Keywords: Host resistance; Zea mays; Fall Army Worm (FAW)

1. Introduction

Maize (*Zea mays* L.) is a major cereal crop feeding 80% of the population of the Central African Republic (CAF) [1]. However, the country is not yet self-sufficient in its maize need and continues to rely partially on imports. Exacerbating this situation is the recent invasive spread of the Fall Armyworm (FAW) since 2016 which has been decimating the maize leading to drastic yield losses [2]. The FAW attacks several crops of economic importance in CAF such as maize, rice, sorghum, millet, cowpea, peanut, sweet potatoes and tomato. The assessment of the FAW infestation situation in CAF showed an incidence ranging from 20% to 80% of attacked feet depending on the localities [2]. The larval stages of the insect have about 300 plants hosts [3]. The ability of the adult stage to fly long distance of about 400 km per night, in part, accounts for their successful spread [4]. The most damaging FAW stage is larvae that feed on leaves, and lateral instars damage every part of the plant [5]. Due to its attack, the plant's photosynthetic area reduces, and it also directly damages the grains [6-7].

Despite the importance of maize as an important food crop for CAF, its average productivity remains low in the country. Productivity is further reduced by the invasion of FAW.

Various control measures continue to be tested globally for the control of FAW, including chemical control [8], biological control using microbial organisms and predatory insects that attack FAW [9], use of genetically modified crops with Bt genes, and integrated pest management [10-12]. However, the use of developing genetic resistance in maize against the pest remains under-explored [13-15]. Induced genetic variation leads to a widened and novel genetic base for selections for traits of interest. This part of work focused on the identification of optimum dose of gamma irradiation treatment on seeds that could be used for improving productivity of maize and developing Resistance to the FAW using Radiation-Induced Novel Genetic Diversity.

2. Materials and Methods

2.1. Choice of study site

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].

The region has a strongly denatured, red ferrallitic soil [16]. The Boukoko regionis covered by the subequatorial and peri-Guinean semi-deciduous forests. Following the strong anthropogenic pressure, some of these forests have disappeared and the initial vegetal landscape of the region has been modified. Thus, the forest is replaced by a vegetation of anthropic origin made up of spontaneous palm groves, grassy savannahs with Panicum maximum, *Imperata cylindrica*, and pre-forest savannahs often dominated by *Chromo-laena odorata* and *Caloconba Caloncaba weluitshii* [17].

2.2. Vegetable material

The test of performance from irradiated seeds was performed on three varieties of maize from Cameroon 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

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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, 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							Yellow			

2.3. Experimental test and measured parameters

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 on May 07, 2021, for the variety CMS 8704 (Plot 1), on May 08, 2021, for the variety CMS 2019 (Plot 2), May 10, 2021 for the CMS 8501 variety (Plot 3) and May 11, 2021 for the local ecotypes (plot 4). 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. 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]:

(a) $$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds planted}} \times 100$$

(b) $$\text{Survival percentage} = \frac{\text{Number of surviving seedlings}}{\text{Number of seeds planted}} \times 100$$

(c) $$\text{Average seedling height} = \frac{\text{Sum of seedling height in mm}}{\text{Number of plants measured}}$$

2.4 Data analysis

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	Replication /Dose	Control	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy
Germination percentage	I	100	100	100	45	35	0
	II	100	85	75	65	20	5
	III	100	95	100	70	30	0
	Mean	**100**	**93.3**	**91.6**	**60**	**28.3**	**1.6**
	Control	**100**	**93**	**92**	**60**	**28**	**2**
Survival percentage	I	100	100	100	10	35	0
	II	100	85	85	60	5	0
	III	100	85	100	45	10	0
	Mean	**100**	**90**	**95**	**38.3**	**16.6**	**0**
	Control	**100**	**90**	**95**	**38**	**17**	**0**
Average seedling height	I	202	200	198	179	124	0
	II	204	202	197	165	89	0
	III	202	181	202	160	103	0
	Mean	**202.6**	**194.3**	**199**	**168**	**105**	**0**
	Control	**100**	**97**	**99**	**84**	**52**	**0**

Statistical analysis of the data obtained was performed with Statistix software. The test of the smallest significant difference at the probability threshold of 5% was used for the comparison of the means of the treatments. Compare the reaction or behavior of seeds irradiated at different doses (100 Gy; 200 Gy; 300 Gy; 400 Gy; 500 Gy) with each other, then with the controls (C), non-irradiated seeds (0 Gy). The graphics were generated using Excel software.

3. Results

3.1. Data from measured parameters CMS 8704 variety

Data from measured parameters CMS 8704 variety are reported in table 2. with 100Gy and 200Gy all seeds were germinated as the control, with 300Gy and 400Gy the half of seed were germinated,with 500Gy 2 to 3 seed of each replicate were germinated, For all dose, there is not attack or symptom of FAW (Table 2; Figure 1).

Table 2: Data from measured parameters CMS 8704 variety

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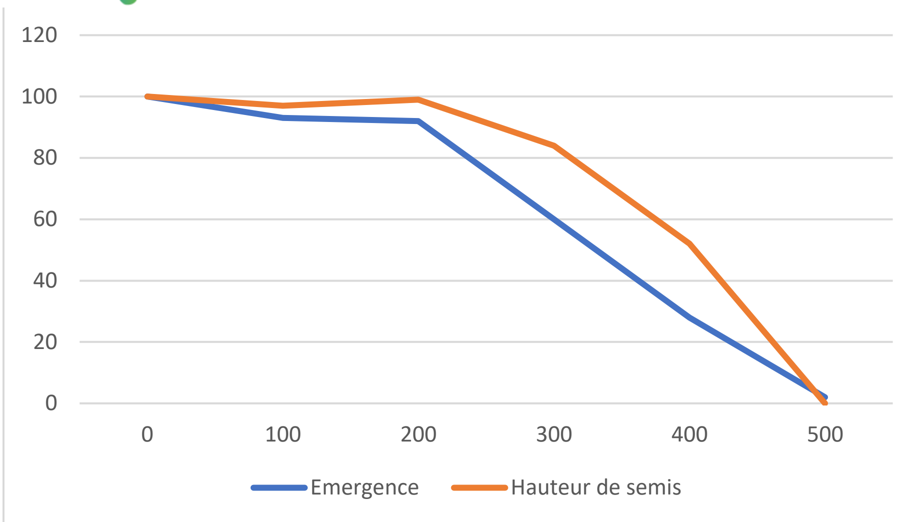

Figure 1. Emergence and seedling height of CMS 87 04 after gamma radiation.

3.2. Data from measured parameters CMS 2019 variety

All seeds from different doses (100Gy, 200Gy and 300Gy) were germinated (Table 3 and figure 2). With 400Gy the half of seed from each replicate were germinated, with 500Gy some replications give 2 to 3 plants. For all dose, there is not attack or symptom of FAW.

Table 3: Data from measured parameters CMS 2019 variety

	Replication / Dose	Contrôle	100 Gy	200 Gy	300 Gy	400 Gy	500 Gy
Germination percentage	I	100	95	100	90	70	10
	II	100	95	100	100	55	0
	III	100	100	95	90	60	15
	Mean	100	96.6	98.3	95	61.6	8.3
	% of Control	100	97	98	95	62	8
Survival percentage	I	**100**	**95**	**100**	**80**	**55**	**10**
	II	**100**	**95**	**100**	**80**	**45**	**0**
	III	**100**	**100**	**90**	**85**	**45**	**10**
	Mean	**100**	**96.6**	**96.6**	**81.6**	**48.3**	**6.6**
	% of Control	**100**	**97**	**97**	**82**	**48**	**7**
Average seedling height	**I**	**203**	**202**	**201**	**178**	**107**	**57**
	II	**202**	**203**	**202**	**189**	**111**	**0**
	III	**202**	**203**	**192**	**178**	**158**	**88**
	Mean	**202.3**	**203.6**	**198.3**	**181.6**	**125.3**	**48.3**
	% of Control	**100**	**100**	**99**	**91**	**63**	**24**

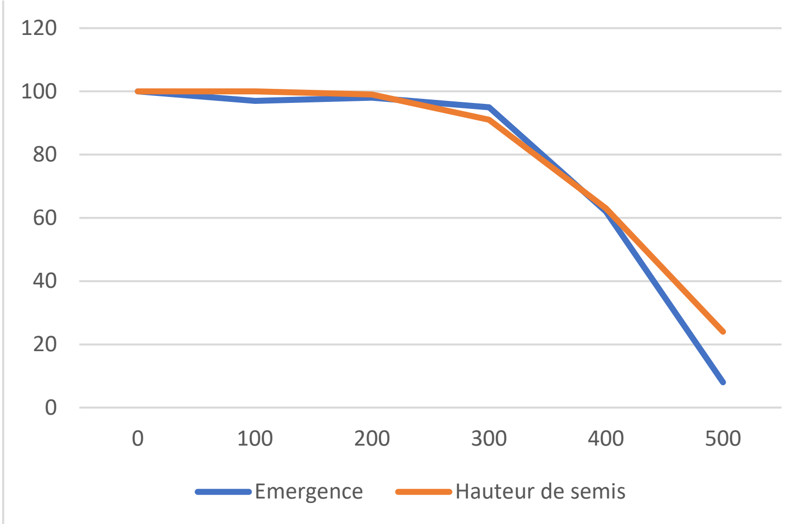

Figure 2. Emergence and seedling height of CMS 2019 variety after gamma radiation.

3.3 Data from measured parameters CMS 85 01 variety

All seeds from diffrents doses (100Gy , 200Gy; 300Gy ; 400Gy and 500Gy) were germinated as the control, but the major of plants are attacked by the FAW (table 4 and figure 3).

Table 4: Data from measured parameters CMS 85 01 variety

	Réplication / Dose	**Contrôle**	**100 Gy**	**200 Gy**	**300 Gy**	**400 Gy**	**500 Gy**
Germination percentage	I	100	95	95	90	75	30
	II	100	90	100	100	60	20
	III	100	95	90	95	60	40
	Mean	100	93.3	95	95	65	30
	% of Control	100	93	95	95	65	30
Survival percentage	I	90	90	90	75	65	20
	II	100	90	90	90	50	5
	III	95	90	75	95	50	40
	Mean	95	90	85	86.6	55	21.6
	% of Control	95	90	85	87	55	22
Average seedling height	I	202	203	202	193	184	85
	II	202	202	200	190	102	85
	III	201	202	200	189	125	73
	Mean	201.6	202.6	200.6	190.6	165.6	81
	% of Control	100	100	100	95	83	40

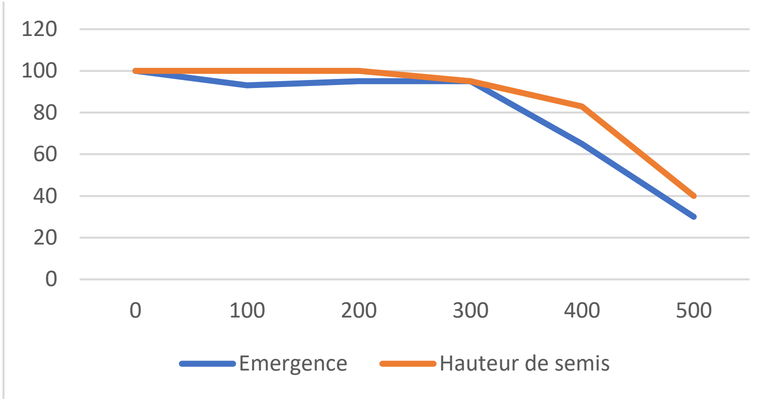

Figure 3. Emergence and seedling height of CMS 85 01 variety after gamma radiation.

3.4 Data from measured parameters of local ecotype

With 100Gy and 200Gy all seeds were germinated as the control, but with hygh dose (300Gy ; 400Gy and 500Gy) the major of seeds were not germinated (Table 5; figure 4) . We don't observe the symptom of FAW (Fall Armyworm).

Table 4: Data from measured parameters local ecotype

	Réplication / Dose	**Contrôle**	**100 Gy**	**200 Gy**	**300 Gy**	**400 Gy**	**500 Gy**
Germination percentage	I	65	65	30	15	5	0
	II	75	45	40	20	5	0
	III	55	70	70	15	15	0
	Mean	65	60	46.6	16.6	8.3	0
	% of Control	65	60	47	17	8	0
Survival percentage	I	65	50	30	15	5	0
	II	75	40	35	20	5	0
	III	60	65	65	10	5	0
	Mean	66.6	51.6	43.3	15	5	0
	% of Control	67	52	43	15	5	0
	I	202	202	202	144	116	0
Average seedling height	II	203	202	202	180	99	0
	III	202	202	200	169	34	0
	Mean	202.3	202	201.3	164.3	83	0
	% of Control	100	100	100	82	42	0

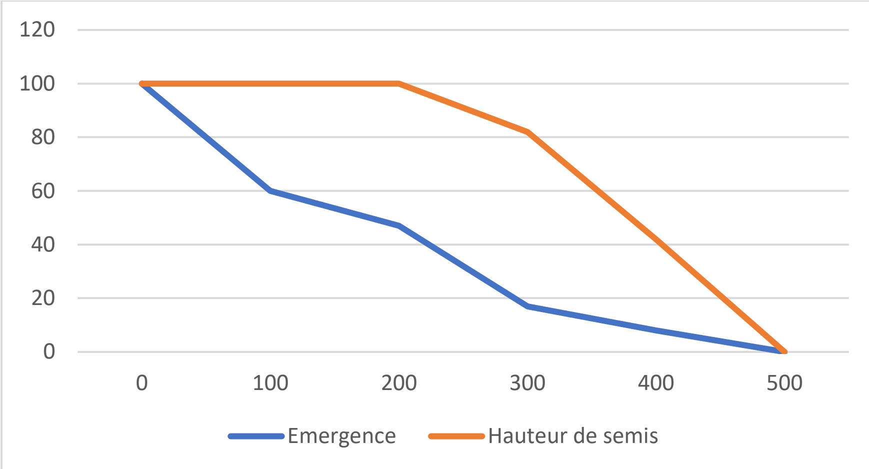

Figure 4 Emergence and seedling height of local ecotype after gamma radiation.

4. **Discussion**

The use of ionizing irradiation has been useful for the genetic improvement crop. However, its use should be preceded by assays that determine the effect that irradiations have on the plant material. A radio stimulant low-dose is defined as any dose from environmental radiation level and the threshold that marks the boundary between positive and negative biological effect [20]. 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 [21-22]. Our results show that fir the CMS 8704 variety with 100Gy and 200Gy all seeds were germinated as the control, with 300Gy and 400Gy the half of seed were germinated, with 500Gy 2 to 3 seeds of each replicate were germinated, but according to the different doses, the CMS 8704 presents no symptom of FAW.

The data from measured parameters local ecotype show that with high dose (300Gy; 400Gy and 500Gy) the major of seeds were decreased plant germination and development. These findings confirm the results obtained by earlier studies that showed the inhibitory effects of plant growth and development exposed to high doses of gamma radiation, simultaneous with the increase of reactive oxygen species generated through water radiolysis [23-25]. Massive doses of ionizing radiation have been shown to induce physiological changes in plants, such as enhancement of respiration, increase in ethylene production and induction of enzyme activities (particularly for phenolic metabolisms and accumulation specific protein species). These effects are considered a consequence of both the direct interactions between the ionizing radiation [26-27].

5. Conclusion

All seeds from diffrents doses (CMS-20 19, CMS8704 and the Local ecotype) were germinated from diffrents doses (100Gy, 200Gy and 300Gy) and did be presented, the symptom of FAW. Howerver f or the CMS85 01, all seeds from diffrents doses (100Gy, 200Gy; 300Gy; 400Gy and 500Gy) were germinated as the control, but the major of plants are attacked by the FAW (Fall Armyworm). Considering the effects of radiation on plants, the present study was conducted to determine the effects of radiation on maize growth and development and on the content of photosynthetic pigments. Furthermore, we used ESR spectroscopy to study the behavior of a radiation-induced free radical in gamma-irradiated maize seeds and to correlate it with the germination pattern.

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