International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 4 No. 4 Dec. 2013, pp. 665-671

http://www.issr-journals.org/ijias/

Radio-sensibility of pearl millet (*Pennisetum glaucum (L.) R. Br.*) and cowpea (*Vigna unguiculata (L.) Walp.*) seeds germination and seedling growth

Sani Daouda Ousmane[1], Wilfred Elegba[2], and Kenneth Danso[2]

[1]Department of Radio-Agronomy and Ecophysiology,
Biotechnology and plant Breeding Laboratory, Radio-Isotopes Institute, AM University,
Niamey, Niger

[2]Biotechnology and Plant breeding Section,
Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission,
Accra (Legon), Ghana

ABSTRACT: Millet and cowpea crops are important for food security, poverty alleviation and malnutrition in the arid Sahel. The two crops generally grow together and have the advantage of supporting poor climatic conditions such as drought and low use of inputs which characterize agriculture in the world's least developed countries. Recent climate change raised up the dryness phenomenon and the traditional species cycle of production is no longer adapted to the reduced length of the wet seasons. New early and drought tolerant varieties are needed to ensure a steady supply of food in the driest regions on the planet. As it was done in numerous species, the mutation induced breeding can help to develop new crop varieties in pearl millet and cowpea. This method is carried out in Niger, in the Laboratory of Plant Breeding and Biotechnology to find out varieties of pearl millet and cowpea, more drought and high temperatures tolerant. This work examines the sensitivity of these two species to gamma radiation, as a first prerequisite step, for crop improving using mutation induction technique. The LD50 was found to be 669,3 Gy for millet crop and respectively 176,6 and 209,4 Gy for TN578 and IT90K 372-1-2 cowpea varieties.

KEYWORDS: Pearl millet, cowpea, seeds irradiation, food security, Niger.

1 INTRODUCTION

Millet and cowpea are important crops in Niger. Millet is the staple food of the majority of the Niger population, the cowpea is a source of cash for farmers especially women. These two crops are usually grown in association and have the advantage of producing where other species would have perished. In millet as in cowpea grain productivity is related to the length of the cycle. The most productive varieties are those that have the longest cycle. Recent climate changes result in shortening the duration of the wet season in the Sahel region, making increasingly unsuitable varieties with long vegetative cycle. The need to create new varieties combining productivity and precocity is an obligatory passage for the achievement of the Millennium Development Goals in nutrition and human health in the Sahel component. Conventional breeding has a relatively high cost for closed secondary interest crops like millet and cowpea. It takes 10 to 15 years of experimentation to lead to new varieties. Selection by induced mutation techniques, though it remains uncertain, has the advantage of saving time and ease of implementation. This technique is carried out in Niger, in the Biotechnology and Crop Improvement Laboratory, to increase pearl millet and cowpea crops drought tolerance. This work is a first step, studying the radio-sensitivity of the two species, to determine lethal dosage (LD_{50}) of gamma ray irradiation. It would, then, be used for irradiating and mutation induction breeding.

Corresponding Author: Sani Daouda Ousmane (dsaniri@yahoo.fr)

2 Material and Methods

2.1 Seeds Irradiation

Seeds of two varieties of cowpea (TN578 and IT90K-372-1-2) and one variety of pearl millet (HKP) coming from the Biotechnology and Crop Improvement Laboratory of National Radio-Isotopes Institute were used in this experiment. Gamma ray irradiation took place in the Radiation Technology Center of Ghana Atomic Energy Commission (GAEC), in Accra in November 2012, using Cobalt-60 source. Dry seeds were split in 8 samples (of sixty five grams), each corresponding to one of the seven irradiation treatments 0 (Control), 100, 200, 300, 400, 500, 600 and 700 Gy. All irradiation was done with a rate of 258,8 Gy/h, determined during a preliminary irradiation. After irradiation, seeds were sown, half *in vitro* in Petri dishes, and half in soil in the green house.

2.2 *In vitro* germination test

In vitro germination test was done in Petri dish of 90 mm diameter with cotton wetted with 10 ml of distilled water for pearl millet and 20 mm for cowpea. Each Petri dish received 10 seeds and there were 10 Petri dishes by treatment for pearl millet and one for cowpea. Incubation took place in dark, in a growth room at ambient temperature of 25 to 36°C. The percent of germination was recorded three days after sowing (DAS).

2.3 *In* soil germination and seedling growth

In soil test took place in a green house, on a germination tray for the pearl millet and in PVC pot for the cowpea. The soil used is a mixture of local topsoil and sawdust in a radio of 2:1 (v/v). Seeds were sown in a small hole of 2 to 4 cm deep after profusely watering, in a complete randomized experiment replicate 48 times for pearl millet and 10 times for cowpea. Spacing was 5 cm from plant to plant in millet and in pot of 10 cm diameter for cowpea. The experiment was irrigated at suitable intervals in such a manner that the crop did not experience with water stress. The data regarding the seedling emergence and plant height were recorded 7 days after sowing. In the result presentation, the seeds controls (with no radiation) were considered as reference (100%) of normal growth.

3 Results and discussion

3.1 Seeds germination and seedling lifting up

Data on the effects of different doses of gamma ray on seeds germination were summarized in Table 1. The two species responded differentially to various gamma radiation doses. For millet crop, the average rate of germination obtained *in vitro* is 98,9% and 104,6% in pot, with no significant difference between treatments, *in vitro* so as in soil, however the lower germination value is for the highest radiation dose. On figure 1, we can easily notice no difference between treatments. Germination is not negatively affected by gamma radiation even at high doses. This result is similar to some previous results [1]. Working on *Lepidium sativum* L. with dosages varying between 0 and 800 Gy, they found that gamma radiation affect significantly all observed important traits except germination percentage. It was concluded that the germination percentage is not a good indicator of radio-sensitivity in pearl millet. In soil test, the germination ability seems to be stimulated by all irradiation doses. Only the latest higher dose of 700 Gy showed inhibitory effect. This result was reported by several authors [2], [3]. In pearl millet, effective radiation doses are higher than 600 Gy, whereas previous results worked exclusively on doses under this value [4], [5]. This result should be considered and radiation doses above 700 Gy should be prospected in pearl millet.

Table 1. In vitro and in soil germination rate of pearl millet and cowpea as affected by radiation doses

Date	CTL	100	200	300	400	500	600	700	Average	STDEV
Pearl millet *in vitro*	100	101,0	100,0	99	100,0	94,9	98,0	99,0	**98,9**	**1,8**
Pearl Millet in soil	100	107,3	104,9	107,3	109,8	102,5	109,8	94,1	**104,6**	**4,8**
Cowpea *in vitro*										
TN578	100	100	100	100	100	80	70	60	**88,8**	**16,4**
V2	100	90	90	90	90	70	60	50	**80,0**	**17,7**
Cowpea *in soil*										
TN 578	100	80	140	20	20	60	20	20	**57,8**	**45,9**
V2	100	112,5	75	25	25	25	25	12,5	**50,0**	**39,5**

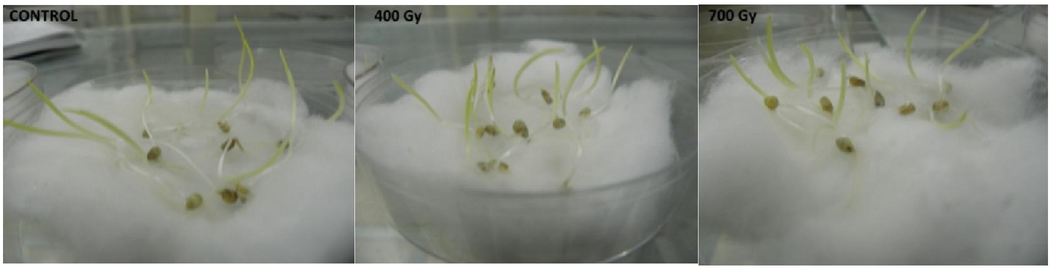

Fig. 1. in vitro Pearl millet (Pennisetum glaucum (L.) R. Br.) germination, three days after incubation, as affected by the dose of seeds' Radiation between 0 to 700 Gy

Instead, cowpea showed mean values of germination rate ranged from 50,0% to 88,8%, with great differences tests and between varieties. In *in vitro* test, inhibitory effects began at higher radiation dose (> 500 Gy), whereas in soil, it started from 300 Gy. The germination rate showed regular decrease with increase in radiation dose. In *in vitro* test, from 100 to 400 Gy, there was no significant effect of radiation. The germination rate began to fall down at 500 Gy, to attend 60% and 50% for TN578 and IT90K respectively at highest dose. In soil test, the inhibitory effect began at 300 Gy and decreased progressively to attend 20% and 12,5% for the highest dose, respectively for TN578 and IT90K varieties (see Figure 2).

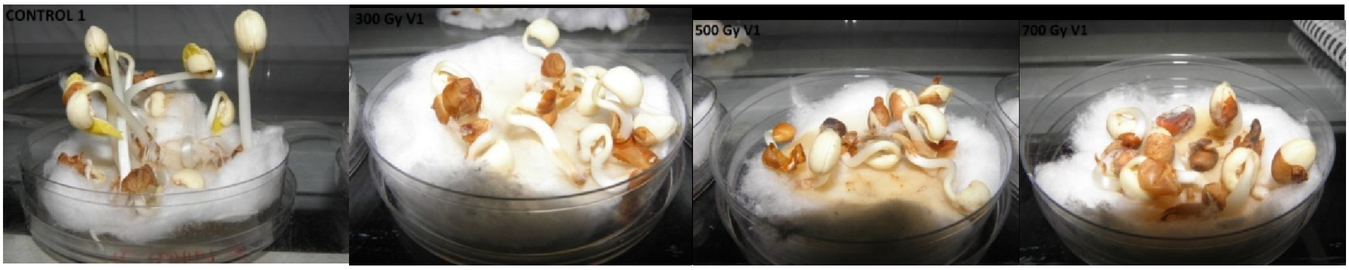

Fig. 2. in vitro cowpea (Vigna unguiculata (L.) Walp.) germination, three days after incubation, as affected by the dose of seeds' Radiation between 0 to 700 Gy

The difference between *in vitro* and *in* soil test indicate that the inhibitory effect affects more the epicotyls and hypocotyls growth (reducing seedling lifting up) than the germination rate.

3.2 SEEDLING GROWTH

Data on the effect of different doses of gamma ray on seedling growth are graphically illustrated in Fig 5 to 8. Lower doses of 200 Gy did not reduce the seedling significantly seedling growth. Higher doses exerted inhibitory effects on seedling growth of both millet and cowpea (Figures 3 and 4). Radiation effect was recorded with higher doses above 300 Gy in pearl millet (Fig 5) and above 200 Gy in cowpea (Fig 7). This response is in great agreement with earlier research findings [4], [6], [7], [5], [8]. This decline in stem height was obtained even in the case of massive inflows of mineral fertilizers [9]. In pearl millet, plant height decrease progressively, with radiation dose, from 100% of the control, to 40% at the highest dose (700 Gy). In cowpea, the height drop is sharper, from 200 to 300 Gy and plant growth is almost zero beyond 300 Gy. No significant difference was recorded between the two varieties of cowpea.

***Fig. 3.** Pearl millet (Pennisetum glaucum (L.) R. Br.) seedling growth in pot, in green house, three days after sowing, as affected by the dose of seeds' Radiation between 0 to 700 Gy.*

***Fig. 4.** Cowpea (Vigna unguiculata (L.) Walp.) Seedling growth in pot, in green house, three days after sowing, as affected by the dose of seeds' Radiation between 0 to 700 Gy.*

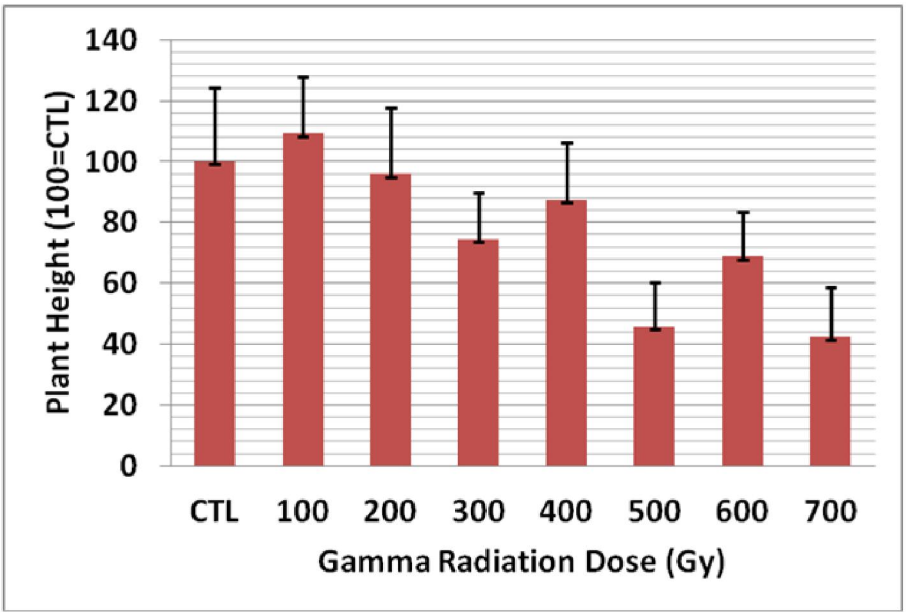

Fig. 5. Pearl Millet (variety HKP) height as affected by gamma radiation dose, 7 days after sowing

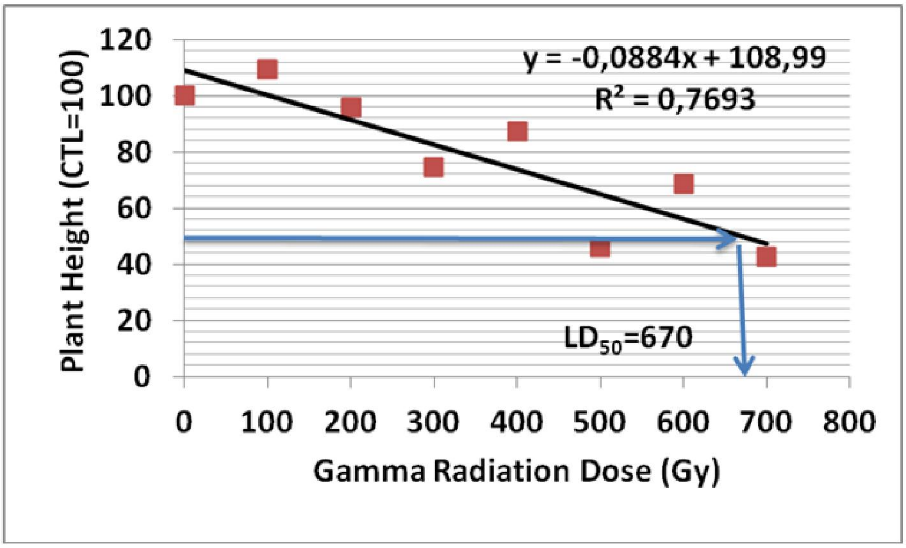

Fig. 6. Pearl Millet (variety HKP) height as affected by gamma radiation dose, 7 days after sowing. LD_{50} determination

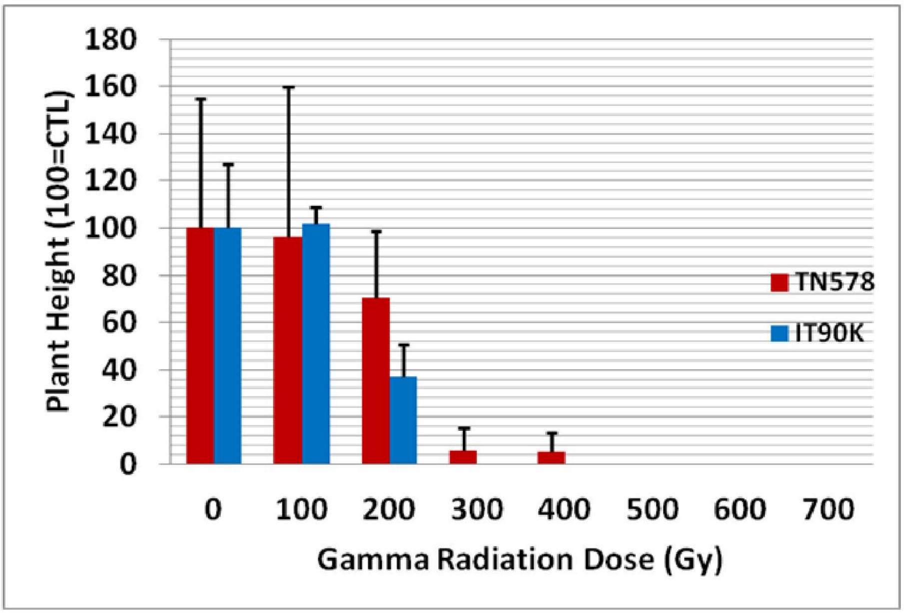

Fig. 7. Cowpea height as affected by gamma radiation dose, 7 days after sowing. Varieties TN578 and IT90K

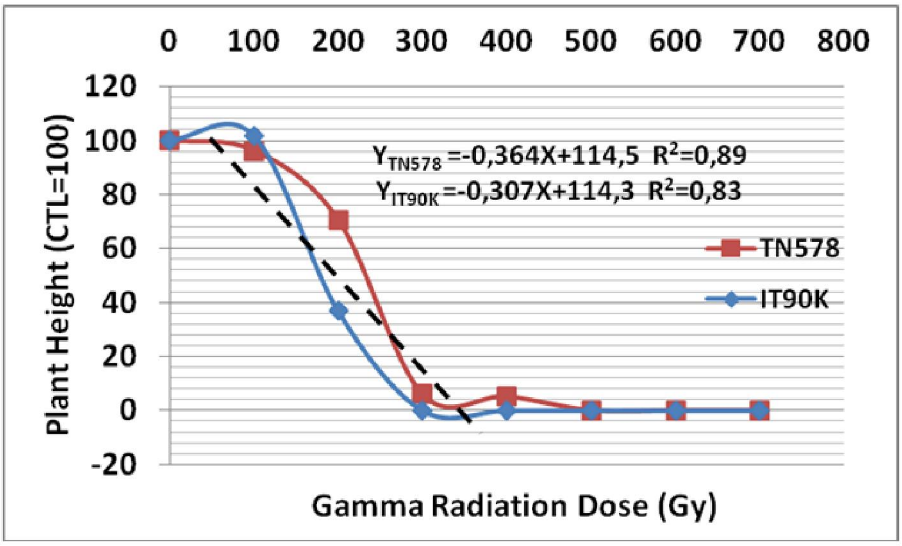

Fig. 8. Cowpea height as affected by gamma radiation dose, 7 days after sowing. Varieties TN578 and IT90K. LD_{50}determination

3.3 Lethal Dose of 50% growth inhibition determination (LD_{50})

The results above permit to consider the seedling height as a good indicator of the biological effect of the gamma rays on seeds and seedling growth of pearl millet and cowpea. The data were used to calculate the Lethal Dose for 50% growth inhibition. The radiation doses showed negative correlation with seedling growth (Fig 6 and 8). As expressed in the equations below, regression analysis revealed that the decrease for unit increase in dose was 0,088 in pearl millet, 0,307 in cowpea TN578 and 0,364 in cowpea IT90K, with regression coefficients varying from 0,77 to 0,89.

$$Y_{MIL} = -0{,}088\,X + 108{,}9 \qquad R^2=0{,}77$$
$$Y_{TN578} = -0{,}307\,X + 114{,}3 \qquad R^2 = 0{,}83$$
$$Y_{IT90K} = -0{,}364\,X + 114{,}3 \qquad R^2=0{,}89$$

Where:

Y is the relative growth in percent

X is the radiation dose in Gray

The LD_{50} is the gamma ray dose expected to produce enough genetic modification to lead to new mutant viable lines. Using the above formula, the LD_{50} is the X value calculated with Y equal to 50. Obtained values were presented in Table 2 : 669,3 Gy for millet; 209,4 Gy for cowpea TN578 and 176,6 Gy for cowpea IT90K. Recently, some authors [5], working on plant mortality as observed indicator, found LD_{50} values varying from 599 to 731 Gy for pearl millet. Even the observed indicators were different, the LD_{50} values obtained were similar for the two experimentations, with different varieties of pearl millet. Anggia [10] working with large game of dosage (0-2000 Gy) on corn, found LD_{50} mean values ranged from 220 to 615 Gy depending on the variety. Instead, LD_{50} is variable between cowpea varieties and was to be determined before working on each new variety of this species.

Table 2. Calculated LD50 for pearl millet and Cowpea in Niger

Crop	LD_{50} (Gy)
Pearl Millet	
HKP	669,3
Cowpea	
TN578	209,4
IT90K	176,6

4 CONCLUSION

The pattern of seedling growth response of pearl millet and cowpea seeds to gamma ray irradiation has been successfully determined. In addition, LD_{50} relative to plant eight of the tested varieties was found to 669 Gy for millet and ranged from 176 to 209 Gy for cowpea. The stimulation positive effects observed in pearl millet, for all tested doses, suggested to further irradiate this specie with gamma radiation dosages above 700 Gy. In the other hand, our results showed that pearl millet is more tolerant than cowpea to gamma ray radiation.

REFERENCES

[1] Abdoul-Majeed, A.U.R. Khan, A. Habib and M. Zahir, "Gamma Irradiation Effects on some Growth Parameters of *Lepidium sativum* L.," *ARPN Journal of Agricultural and Biological Science*, vol. 5, no. 1, January 2010.

[2] H.M. Badr, A.A Alsadon, and A.R. Al-Harbi, "Stimulative Effect of Gamma Radiation on Growth and Yield of Two Tomato (*lycopersicon esculentum,* Mill) Cultivars," *J. King Saud. Univ. Agric. Science,* vol. 9, no. 2, pp. 277-286, 1997.

[3] J. Sumira, P. Talap, T. Siddiqi, and Mahmooduzzafar, "Gamma radiation effects on growth and yield attributes of *Psoralea corylifolia* L. with reference to enhanced production of psoralen," *Plant Growth Regulation,* vol. 64, no. 2, pp. 163-171, June 2011.

[4] R.M. Aslam and K.A. Siddiqui, "Cytogenetic studies of variability induced through hybridization and gamma irradiation in *Pennisetum americanum* I. seedling emergence and plant survival in M1 generation," *Pak. J. Bot.,* vol. 11, no. 2, pp. 173-177, 1979.

[5] L.N. Horn, M.B.A. Chikelou, B. Soleymanne, and S.N.A. Ipinge, "Radiosensitivity studies in pearl millet (*Pennisetum glaucum*), Cowpea (*Vigna unguiculata*) and Sorghum (*Sorghum bicolor*) varieties in Namibia," *AGRICOLA* 2010, pp. 38-40, 2010.

[6] H. Khizar, A. Khan, M. Sadidg, F. Elahi, and A. Shakoor, "Gamma Radiation Induced Variation in Sorghum Cutivars," *Pakistan J. Agric. Res.,* vol. 11, no. 1, 1990.

[7] H.N. Abla, M.F. Hashim, N.S. Hassan, and H. Abo-Zaid, "Effect of Gamma Irradiation and Phosphorus on Growth and Oil Production of Chamomile (*Chamomilla recutita* L. Rauschert)," *Int. J. Agri. Biol.,* Vol. 6, No. 5, 2004.

[8] M.M. Khan, R. Din, M.M. Qasim, N. S. Jehan, and M.M. Iqbal, "Induced Mutability studies for yield and yield related characters in three wheat (*Triticum aestivum* L.) varieties," *Asian Journal of Plant Sciences* 2 (17-24) 1183-1187, 2003.

[9] F. Subhan, M. Anwar, N. Ahmad, A. Gulzar, A.M. Siddiq, S. Rahman, I. Ahmad, and A. Rauf, "Effect of Gamma Radiation on Growth and Yield of Barley under Different Nitrogen Levels," *Pak. J. Biol. Sci.,* 7(6) : 981-983, 2004.

[10] E.P. Anggia, D. Fajar, H. Kustanto, and D. Ruswandi, "Study on LD_{50} of Gamma Rays Irradiation (CO^{60}) for mutation of Unpad corn collections," *Zuriat,* Vol. 21, No. 1, January 2010.