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Full Length Research Paper

Radio-sensitivity of selected cowpea (*Vigna unguiculata*) genotypes to varying gamma irradiation doses

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An appropriate dose of radiation should be established on target genotypes before large scale mutagenesis undertaken. The objective of this study was to determine an ideal dose of gamma radiation to induce genetic variation in selected cowpea (*Vigna unguiculata*) genotypes. Seeds of three Namibian released cowpea genotypes were gamma irradiated using seven dozes at the International Atomic Energy Agency, Austria. Experiments were laid out in the completely randomised design with three replications and important data collected. Data were subjected to analysis to identify optimal lethal dose aiming LD_{50}. Results revealed that genotype Nakare tolerated the radiation dose of 200 Gy providing germination of 43.33%. Seeds of genotypes Nakare and Shindimba failed to germinate above 400 Gy. However, genotype Bira showed germination of 46.67% at 600 Gy, the highest dose used in the study. The optimum doses at LD_{50} for genotypes Nakare and Shindimba are at 150 and 200 Gy, respectively while genotype Bira tolerated increased dose of 600 Gy. Using linear regression model, the LD_{50} for genotypes Nakare, Shindimba and Bira calculated at 165.24, 198.69 and 689 Gy, respectively. The findings may assist as reference doses for large-scale gamma irradiation of cowpea genotypes to induce genetic variation.

Key words: **C**owpea, gamma radiation, LD_{50}, radio sensitivity, *Vigna unguiculata.*

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp., 2n=2x=22) is one of the important food legumes and a useful component of the traditional cropping systems in the semiarid tropics (Ayisi et al., 2000; Singh et al., 2002). Cowpea adapts to harsh environments including extreme temperatures, drought and poor soil fertility. In poor environments it yields comparatively better than other food legumes (Shimelis and Shringani, 2010). The crop originated and domesticated in Southern Africa, which was later spread to east and West Africa and Asia (International Institute for Tropical Agriculture [IITA], 2004). Southern Africa including Namibia, Botswana, Zambia, Zimbabwe, Mozambique and the Republic of South Africa is reportedly considered the center of diversity of *V. unguiculata* where the primitive and wild relatives are found (Ng and Marachel, 1985).

It is reported that at least 95% of farmers in northern Namibia grow cowpea, pearl millet and sorghum. In the country, cowpea ranks second after pearl millet making a crop of importance in the agricultural system. However, cowpea productivity is generally low (250 to 350 kg/ha) since farmers grow unimproved landraces as a result of

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unavailability of improved and locally adapted cultivars. Further, poor cultural practices, insect pest infestation and photoperiod sensitivity contribute to low productivity. The crop is susceptible to a number of fungal, bacterial, and viral diseases and such stress factors are considered to be the major production constraints of cowpea in Namibia (Thottappilly and Rossel, 1992).

In Namibia, since the early nineties, several research activities have been conducted involving cowpea adaptation trials by the Ministry of Agriculture, Water and Forestry in collaboration with the IITA. Consequently, three introduced varieties were released during 1993. However these varieties are less-preferred by farmers due to their proneness to damages caused by insect pests such as aphids, thrips and storage pests particularly weevils. The yield level in the country is below the achievable yield of 1500 to 3000 kg/ha such as reported in Egypt and Malawi (Adeola et al., 2011). Therefore, there is utmost need of cowpea germplasm development and enhancement towards high yield, insect and pest resistance, and drought tolerance in the country.

Despite the rich germplasm collections available by various national breeding programs and the IITA, the genetic base for most self-pollinating crops including cowpea is narrow for economic traits such as grain yield, yield components, drought and insect pest tolerance (Mudibu et al., 2012). Mutation breeding is helpful in pre-breeding or genetic enhancement aimed to develop suitable germplasm. Artificial mutagenesis may bring about fast and direct results to select useful traits unlike the conventional methods in which up to ten years of selections after extensive crosses are required in genetic advancement (Novak and Brunner, 1992). Mutations are the ultimate source of genetic variation, and provide unique germplasm, the raw material for plant breeders (van Harten, 1998). Mutation breeding has been used for generating genetic variation and breeding new varieties during the past decades (van Harten, 1998; Ahloowalia et al., 2004; Tambe and Apparao, 2009). Recently the technique is being applied to generate mutants with altered agronomic traits for genetic studies and to predict the gene function through identification of an allelic series by Targeting Induced Local Lesions IN Genomes (TILLING) (Till et al., 2003; Xin et al., 2008).

Mutations can be induced in various ways, such as exposure of plant propagules, including seeds, tissues and organs, to physical and chemical mutagens (IAEA, 1977; Mba et al., 2010). Physical mutagens are mostly electromagnetic radiations such as gamma rays, X-rays, UV light and particle radiation including fast and thermal neutrons as well as beta and alpha particles. Chemical mutagens include ethylmethanesulfonate (EMS), ethidium bromide, and base analogues such as bromouracil (van Harten, 1998; Girija and Dhanavel, 2009; Mba et al., 2010).

Gamma irradiation is one of the main physical mutagens used to induce genetic variation. The effectiveness of a mutagenic treatment in inducing genetic variations in crop plants depends on the genetic constitution of test varieties and treatment dose, among others (van Harten, 1998; Mba et al., 2010). High doses of mutagenic treatment may destruct growth promoters, increase growth inhibitors and metabolic status of the seed and induce various chromosomal aberrations. Thus high radiation doses would be lethal rendering few plants for selection. This in turn limits the success of artificial selection in the subsequent mutation generations to identify useful mutants. Conversely, low radiation dose are accompanied by early emergence, increased percent germination and field survival with healthy and vigorous seedlings. However, this would possibly be associated with low mutation frequency with reduced selection response towards target mutants. Van Harten (1998) reported that normally genetic mutations occur spontaneously at low frequencies (10^{-5} to 10^{-8} per locus). Limited data is available that evaluated the response of different cowpea varieties at various gamma irradiation doses. Therefore, an appropriate dose of radiation should be established on target genotypes before large scale mutagenesis is undertaken (Tshilenge-Lukanda et al., 2012).

Radio sensitivity or determination of the optimum dose of radiation is a term describing a relative measure of the quantity of recognizable effects of a radiation exposure on the irradiated material (Owoseni et al., 2007). Optimizing the dose of radiation is the first step in induced mutation breeding where its predictable value guide the researcher in the choice of the ideal dose depending on the plant materials and desired outcome. Brown (2013) outlined some negative effects of radiation overdoses such as deletions of DNA nucleotide sequences that may cause reading-frame shifts, inactive protein products, or faulty transcripts. This would subsequently lead into null mutations, in which a particular gene may be inactivated.

According to Mba et al. (2010) the dose of mutagen that is regarded as the optimal is one that achieves the optimum mutation frequency. The LD_{50} is an important parameter to measure the short-term poisoning potential (acute toxicity) of a treatment and widely used to determine the optimum mutation frequency with least possible unintended damage (Owoseni et al., 2007). The optimum dose is obtained through application of different radiation strengths until the LD_{50} is determined. Meyer (1996) described that the LD_{50} is given at once and causes death of 50% of the test material. Even though the LD_{50} value is regarded as the best optimum guideline to determine irradiation doses, Mba et al. (2010) underscored that the range of doses around this level is important for various reasons.

Tshilenge-Lukanda et al. (2012) described that the optimum mutation doses can be determined by recording the percentage seed germination, epicotyl and hypocotyl lengths, among others. In seed propagated crops such as

Table 1. Analysis of variance on percent germination, epicotyl and hypocotyl lengths among three cowpea genotypes tested using seven irradiation doses and three replications.

Source of variation	DF	Germination %		Epicotyl length		Hypocotyl length	
		Mean Square	F value	Mean Square	F value	Mean Square	F value
Replication	2	350.00	3.47^{ns}	0.18	0.64^{ns}	1.69	1.27^{ns}
Genotype	2	14551.03	144.31**	8.63	30.86**	21.15	15.78**
Dose	6	6746.39	66.91**	6.76	24.17**	40.47	30.177**
Genotype*Dose	12	1038.95	10.30**	1.29	4.62**	3.77	2.81**
Error	40	100.83		0.28		1.34	
Total	62						

DF=degrees of freedom; ** denote significant differences at 1% probability level; ns=not significant.

cowpea, Mba et al. (2010) suggested preliminary ranges of gamma irradiation doses of 0 to 600 Gy that should be tested to determine the optimal treatment condition on test genotypes. However these studies did not report an optimal dose of recommendation for cowpea due to differences in genotypic response to the treatment. In groundnut, Tshilenge-Lukanda et al. (2012) tested varied radiation doses of 0, 100, 200, 400 and 600 Gy to determine the optimum dose for mutagenesis.

Research and development collaboration was initiated on mutation breeding in 2009 between the Namibian Government and the International Atomic Energy Agency (IAEA). This created a platform to further develop pre-breeding and breeding of high yielding and drought resistant genotypes of cowpea and cereals such as pearl millet [*Pennisetum glaucum* (L.) R. Br.; 2n=2x=14] and sorghum (*Sorghum bicolor* L. Moench; 2n=2x=20). The project focused on improving selected crops using induced mutation breeding techniques especially gamma irradiation. Gamma irradiation was recommended by the Namibian Radiation Regulatory Authority as a better option without any impact on the environment. Once the seed is irradiated under a controlled environment mutants can be assayed without radiation contamination. Therefore, the objective of this study was to determine the ideal dose of gamma radiation to induce genetic variation in selected cowpea (*V. unguiculata*) genotypes.

MATERIALS AND METHODS

Plant material and study site

The study used seeds of three Namibian released cowpea genotypes, Nakare (IT81D-985), Shindimba (IT89KD-245-1) and Bira (IT87D-453-2). The genotypes have unique seed shape and colour as well as hilum pattern. Dry, healthy and quiescent seeds were prepared for irradiation. Preliminary germination and viability tests were conducted and provided 100% germination.

The study was conducted at the International Atomic Energy Agency (IAEA), Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria, through a dedicated fellowship grant to the first author under the Technical Cooperation Project (TCP) NAM5009/10 between the IAEA and the Namibian Government.

Gamma irradiation

Thirty seeds per genotype were gamma irradiated in three replications using the gamma irradiation facility at the IAEA. The study used seven irradiation doses (0, 100, 200, 300, 400, 500 and 600 Gy). The 0 Gy dose served as a comparative control. The seeds were packed in separate seed envelopes and placed in desiccators for three days to attain the desired moisture level of 8%. Irradiation was applied using a CO_{60} source Gammacell Model No. 220. The various doses were used to establish the optimum irradiation level that can achieve optimum mutation frequency with least possible and unintended damage (Mba et al. 2010).

Growing plants, experimental design, data collection and analysis

The radio sensitivity (the biological effects of the mutagen treatments on plants) was studied following the methods described by Mba et al. (2010) and Tshilenge-Lukanda et al. (2012). Irradiated seeds were planted in seedling trays with a medium that consisted peat, sand and vermiculate at a ratio of 2:1:1, respectively. Trails were established under environmentally controlled greenhouse with temperatures of 22 to 35°C and light regime kept at 12 h photoperiod.

The experiment was set up in the completely randomised design with three replications. Seedlings were watered twice per week to ensure adequate soil moisture. Seven days after planting germination was recorded and expressed in percentage. Lengths of epicotyl and hypocotyl were measured 14 days after planting. These variables are regarded as suitable indicators in estimating the damage caused by mutagenic treatments. The epicotyl height was measured above the soil surface to the tip of the primary leaf using a ruler and expressed in centimetres. Data were subjected to the standard analysis of variance procedure using Genstat version 11 (Payne et al., 2008) statistical package to compare genotypes and identify the optimal lethal dose aiming LD_{50}. The LD_{50} for each genotype was estimated through the simple linear regression model by fitting the straight line equation $y= mx+c$; where y is the response variable (percent germination), x is the independent variable (irradiation dose), while m and c represent the slope and constant, respectively.

RESULTS

Table 1 summarizes the analysis of variance on percent germination, epicotyl and hypocotyls lengths between

Table 2. Mean and standard deviation on percent germination, epicotyl and hypocoyl lengths among three cowpea genotypes tested using seven irradiation doses.

Genotype	Irradiation dose (Gy)	Parameters		
		Germination (%)	Epicotyl length (cm)	Hypocotyl length (cm)
Nakare	0	100.00±0.00	3.11±0.76	6.71±2.50
	100	70.00±20.00	3.06±0.51	6.37±2.09
	200	26.67±23.09	1.35±1.31	1.90±1.77
	300	0.00±0.00	0.00±0.00	0.00±0.00
	400	0.00±0.00	0.00±0.00	0.00±0.00
	500	0.00±0.00	0.00±0.00	0.00±0.00
	600	0.00±0.00	0.00±0.00	0.00±0.00
Shindimba	0	100.00±0.00	2.79±0.22	5.19±0.62
	100	56.67±15.26	2.30±0.16	4.66±0.80
	200	43.33±15.26	2.21±0.47	3.96±0.88
	300	30.00±0.00	1.26±1.01	0.79±1.06
	400	0.00±0.00	0.00±0.00	0.00±0.00
	500	0.00±0.00	0.00±0.00	0.00±0.00
	600	0.00±0.00	0.00±0.00	0.00±0.00
Bira	0	100.00±0.00	2.86±0.06	5.69±1.46
	100	90.00±10.00	2.94±0.49	6.19±1.76
	200	93.33±11.56	2.75±0.28	4.98±0.37
	300	93.33±11.56	2.75±0.28	3.54±0.28
	400	76.67±15.28	2.22±0.12	3.28±1.69
	500	63.33±15.28	1.88±0.87	3.36±1.86
	600	46.67±11.55	1.82±0.59	0.67±0.29
Mean	0	100.00±0.00	2.46±0.63	5.86±0.62
	100	72.22±19.86	2.92±0.52	5.74±1.64
	200	54.44±33.58	2.77±0.51	3.62±1.69
	300	41.11±41.67	2.10±0.94	1.44±1.7
	400	25.56±39.09	1.33±1.30	1.09±1.84
	500	21.11±32.58	0.74±1.11	1.12±1.92
	600	15.56±24.04	0.63±1.04	0.22±0.36
Grand mean		47.14	1.58	2.73
R^2 (%)		93.90	83.6	81.2
CV (%)		21.30	33.49	42.4

cowpea genotypes, radiation dose and their interaction. A significant (P<0.01) interaction occurred between genotypes and irradiation doses suggesting differential responses of cowpea varieties for the tested irradiation doses.

The mean and standard deviation of percent germination, epicotyl and hypocotyl lengths are presented in Table 2. Germination % decreased drastically in all the three varieties with increased Gy doses (Table 2). Germination was not observed for both Nakare and Shindimba above 300 Gy and 400 Gy, respectively. Genotype Bira could withstand the radiation doses of up to 600 Gy and displayed 47% germination at this dose (Table 2).

The germination response of Nakare, Shindimba and Bira against irradiation doses are given by the linear equations: $y=-0.17x + 78.09$, $y=-0.16x + 81.79$ and $y=-0.08x + 105.12$, respectively (Figure 1). Aiming germination response, y, at 50 the LD_{50} values of genotypes Nakare, Shindimba and Bira were calculated at 165.24, 198.69 and 689 Gy, respectively (Figure 1). The higher the LD_{50} value, the stronger is the resistance shown by the test variety to irradiation and therefore relatively high dose is needed to induce mutagenesis and isolate mutants from the 50% surviving plants.

The overall mean summarised in Table 2 suggests that radiation dose of $\geq$ 300 Gy rendered relatively low germination. The variation in germination was explained by 94% (R^2=93.90) due to genotypic differences and radiation doses (Table 2). The coefficient of variation on percent germination was estimated at 21.2% which is relatively low compared to CVs of lengths of epicotyl and

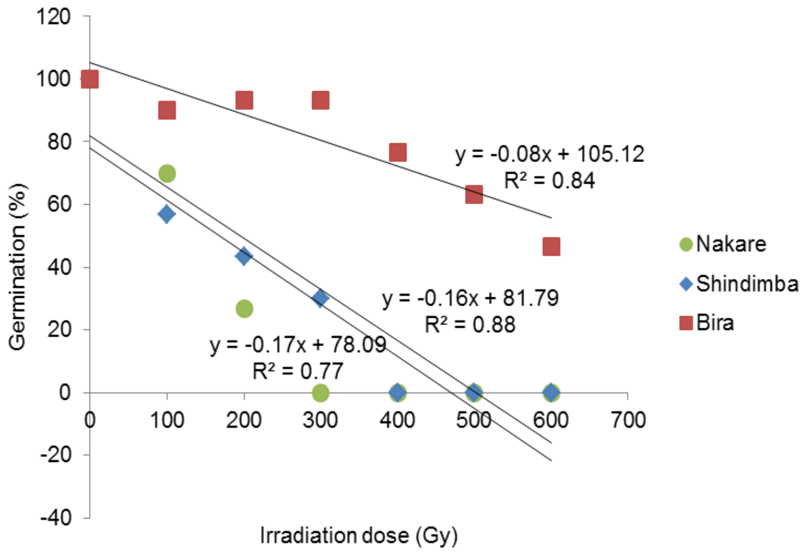

Figure 1. Germination % and fitted straight lines to estimate the LD_{50} in three cowpea genotypes when subjected to seven gamma radiation doses.

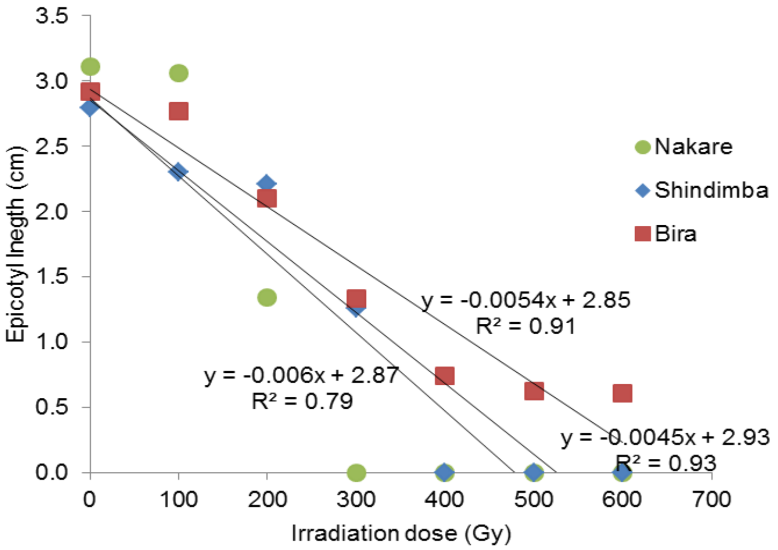

Figure 2. Epicotyl length (cm) and fitted straight lines of three cowpea genotypes when tested by seven gamma radiation doses.

hypocotyl.

In genotypes Nakare and Shindimba both epicotyl and hypocotyl lengths significantly reduced when applying gamma radiation above 200 Gy when compared to the control (Table 2). At the 0 Gy level Nakare showed the highest epicotyl and hypocotyl lengths at 3.11 and 6.71 cm, receptively. Data shown that the lengths of epicotyl and hypocotyl were significantly short in Shindimba and Bira when compared to Nakare at 0 Gy. It appears that in Bira the radiation dose of 100 Gy rendered relatively increased epicotyl length at 2.94 cm and hypocotyl length of 6.19 cm in comparison with the control which recorded 2.86 and 5.69 cm, respectively. The straight line equations showing the trends of the epicotyl and hypocotyl lengths against the seven gamma irradiation doses of the three genotypes are shown in Figures 2 and 3. As expected, the epicotyl and hypocotyl lengths showed decreasing trend as the gamma irradiation doses increased. The coefficient of determination (R^2) estimated in the straight lines were considerably high ranging from 76 to 93% suggesting notable association between the reduction of epicotyl and hypocotyls lengths due to

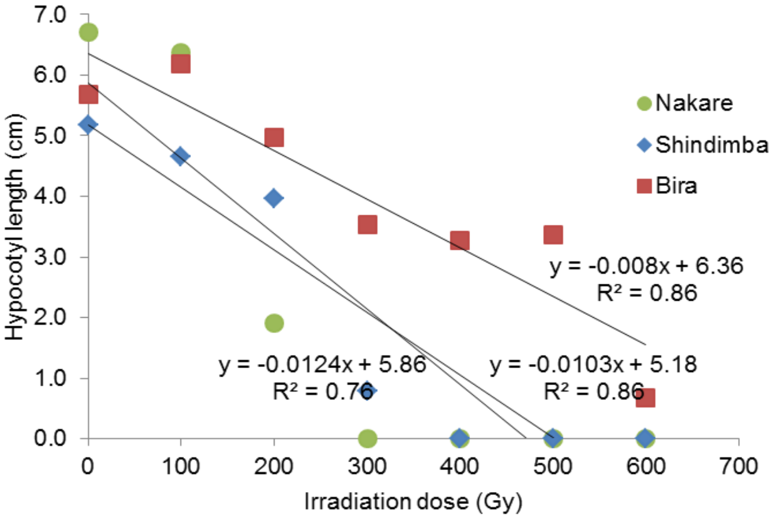

Figure 3. Hypocotyl length (cm) and fitted straight lines of three cowpea genotypes when tested by seven gamma radiation doses.

increased radiation doses (Figures 2 and 3).

DISCUSSION

The present study compared the responses of three Namibian grown cowpea genotypes using seven gamma radiation doses to establish the LD_{50} and to determine associated effects on early growth characters. Results revealed that seed germination, epicotyl and hypocotyl lengths decreased substantially with increased gamma radiation dose. The germination % dropped from 100% (at 0 Gy, control treatment) to 0% when applying 300 and 400 Gy to genotypes Nakare and Shindimba, respectively (Figure 1). The decrease was proportional to the increased dose on the two genotypes. Bira tolerated the doses up to 600 Gy providing germination of 47%. This genotype was more resistant to doses of 200-300 Gy in comparison with Nakare and Shindimba. Mudibu et al. (2012) described that heavy doses of the radiation treatment is associated with toxicity and leads to undesirable changes including chromosomal aberrations, lethality, injury, and sterility. These anomalies are measured as the reduction in germination, survival, plant growth and fertility as well as increase in frequency of chromosomal aberrations and chlorophyll deficient chimeras.

This study found that the LD_{50} for genotypes Nakare, Shindimba and Bira were achieved at 165.24, 198.69 and 689 Gy, respectively. Nakare required low gamma irradiation dose to achieve the expected LD_{50} comapred to Shindimba and Bira. Conversely, genotype Bira was the most tolerant to heavier dose of radiation and only reached to the desired LD_{50} at 689 Gy. Mba et al. (2010) and Owoseni et al. (2007) described that the irradiation level for generating mutants in crop improvement programmes should be carried out within a range of ± 5 units of the experimentally determined optimal dose.

Further, the present findings showed that there has been a progressive reduction in the mean height of epicotyl and hypocotyl in all genotypes as the radiation dose increased. Manju and Gopimony (2009) pointed out that the reduction in the survival of plants is an index of post germination mortality resulting from cytological and physiological disturbances due to the effect of irradiation. Decreased plant height and growth was also observed in a similar experiment on rice varieties in Sierra Leone (Harding et al., 2012). The authors indicated that the percentage survival of germinated seedlings decreased significantly within 8 to 14 days with increase in radiation doses up to 600 Gy in a laboratory condition. According to Sparrow and Evans (1961) the reduction in lengths of the epicotyl and hypocotyl could be attributed to the destruction of the plant growth hormone, auxin, and possibly influenced by the ionizing radiation causing genetic loss due to chromosomal aberration. Summarily, the current study confirmed that varied doses of gamma radiation applied on three different cowpea genotypes differentially affected germination, and early growth and development significantly. Experimentally selected dose of the gamma radiation may help as a generic treatment dose to induce large scale mutagenesis in cowpeas.

Conclusion

Based on the differences between the irradiated and non irradiated plant materials, different germination, epicotyls and hypocotyls length were observed. Through this study the doses leading to an average of 50% damage (LD_{50})

to seed germination in genotypes Nakare, Shindimba and Bira varieties were determined. These baseline doses are important for large scale mutagenesis and to increase genetic variation among crop varieties such as in cowpea. The result demonstrated that cowpea genotypes required specific irradiation dose to undertake large-scale mutagenesis. It should, however, be taken into consideration that induced mutations are random events and as such published irradiation conditions might not result in the same mutation events for different genotypes.

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