Indian Journal of Agricultural Sciences **85** (9): 1148–52, September 2015/Article
https://doi.org/10.56093/ijas.v85i9.51564

Effects of gamma irradiation on germination and physiological parameters of maize (*Zea mays*) genotypes

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Received: 5 January 2015; Accepted: 9 June 2015

ABSTRACT

This study determined the effect of gamma irradiation on the seedling growth parameters and enzymatic activities of gamma irradiated seeds of two maize (*Zea mays* L.) genotypes. Twelve doses of gamma irradiation ranging from 0.0025 to 2.0 kGy were given to the seeds with the help of ^{60}Co γ-radiation source facility available at NRL, IARI, and New Delhi. Water activity, germination per cent, seedling growth, dry weight of seedlings and enzymatic activity were determined by using the standard procedures and protocols. Data obtained showed that the seedling length increased in lower doses (0.0.005 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. Water activity of irradiated seeds did not change significantly.

Key words: Alpha amylase activity, Irradiation, Seedling vigor index

Many a times the shortage of water under rainfed ecologies negatively affects of the establishment of maize (*Zea mays* L.) crop (Bahador *et al.* 2015). Several techniques have been involved to increase the germination of different crops under water stress environment. One of such techniques is gamma irradiation. There are some reports in wheat (Mongi Melki and Marouni 2010), sorghum (Mehlo *et al.* 2013) and rice (Sasikala and Kalaiyarasi 2010). Crops which establish that seed germination and seedling vigor improved due to gamma irradiation treatment. But such information is not available for maize. Water activity is an important physical as well as chemical phenomenon in water content of the organic substances. Water activity is a method to determine the availability of water in an organic substance and it also determines the longevity of seed (Gustavo *et al*. 2008). Nuclear techniques, in contrast to conventional breeding techniques are widely applied in agriculture for improving genetic diversity. Unlike conventional breeding procedures which involve the production of new genetic combinations from already existing parental genes, nuclear technology causes exclusively new gene combinations with high mutation frequency. Basic tool of nuclear technology for crop improvement is the use of ionizing radiation which causes induced mutations in plants. These mutations might be beneficial and have higher economical values. Gamma radiation could be beneficial for the alteration of physiological characters of plants (Borzouei *et al.* 2010, Khawar *et al*. 2010). In plant improvements, the radiation of seeds may cause genetic variability that enables breeders to select new genotypes with improved grain yield and quality (Noreen and Ashraf 2009). The effects of gamma irradiations on biological system are because of their interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kazama *et al.* 2008, Afrasiab *et al.* 2012). These radicals can damage or modify the crucial components of the plant cells and influence the morphology, anatomy, biochemistry and physiology of plants depending upon the irradiation dose (Kiong *et al*. 2008).The present study aimed at to ascertain the effect of gamma irradiation on germination, biochemical changes on the treated seeds and its effect on the emergence and on the seed vigor of two maize genotypes.

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MATERIALS AND METHODS

In this study two genotypes of maize, viz. HM 4 and HQPM 1, which are very popular were selected for the study. HM 4 maize genotype was developed by the crossing of maize parents KHI 1105 × HKI 323 and this genotype is adapted and recommended for the *kharif* and rabi seasons. The second maize genotype HQPM-1 was developed by the crossing of HKI 193-1 × HKI 163 parents. This genotype is adapted and recommended for *kharif* as well as rabi seasons. The seeds of these two widely cultivated genotypes of maize were brought from National

Seed Corporation of India, New Delhi.

Seeds of both the genotypes were treated with varied doses of gamma irradiation. The source of gamma irradiation treatments was cobalt-60.These doses (13) were: control (no treatment), 0.0025, 0.005, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.00 and 2.00 kGy.

Water activity of the gamma irradiated seeds was carried out (3 replications) with the help of water activity meter (Hydrolab 3 Rotronic ag). Water activity meter works on the following principle.

$a_w = \rho/\rho_0$ and equivalent to relative humidity and RH is expressed in percentage

ERH% = $a_w \times 100$, Gustavo *et al*. (2008)

Irradiated 30 seeds were mounted in the germination paper with suitable moisture to germinate. Mounted seeds for germination were kept in an incubator at 22ºC for seven days. The germination counting and temperature (ºC) were monitored on daily basis and finally germination per cent was calculated on the seventh day.

Germination percentage after emergence of seedlings was calculated by the using formula as follows:

Final Germination Percentage (FGP) = (NT × 100)/N

where NT is the proportion of germinated seeds in each treatment for the final measurement, N= Total number of seeds used in each treatment of gamma irradiation (Anjum and Bajwa 2005). The seedling length was measured with the help of a standard scale.

Dry weight (g) of seedling was determined after 72 hr putting the seedling in the oven at 65^{0}C. Seedling vigor index was calculated by formula as given below

Seedling vigor index I = Per cent germination × plant height (cm) (Maisuria and Patel 2009).

Alpha amylase enzymatic activity was determined as per the standard protocol. Absorbency spectrophotometer (UV-2450 SHIMADZU Corporation) was used for determining the absorbance and the observations were taken at 450 nm wavelength (Maryam *et al.* 2008).

RESULTS AND DISCUSSION

Water activity of any organic substance is an important entity to decide its moisture. Gamma irradiated maize genotypes seeds were subjected to determine water activity. Data gathered for water activity were analyzed and it is evident that it did not change significantly with increasing gamma irradiation doses (Fig 1).

Data gathered for germination percent at different doses of gamma irradiation for HQPM 1 as well as HM 4 was assessed and it is found that the germination per cent of HQPM 1 was significantly more than HM 4 genotypes. Higher germination percentage of HQPM 1 than HM 4 might be due stimulating effects on activating RNA or protein synthesis of HQPM 1 maize genotype (Fig 2). Germination per cent for HM 4 maize genotype did not change significantly upto 0.2 kGy dose beyond this dose it reduced at 0.3 kGy by 12.5% and the maximum reduction was recorded at 2.0 kGy by 50% (Fig 2). Similarly germination

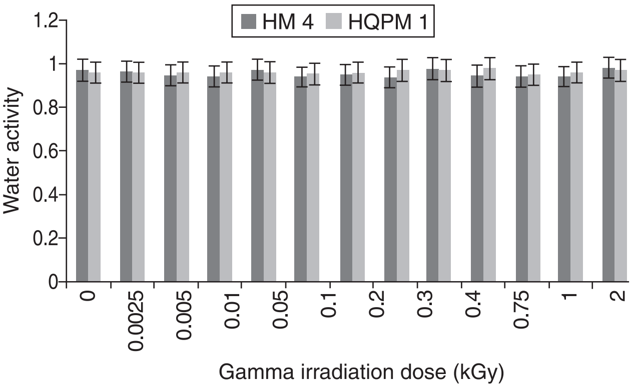

Fig 1 Effect of gamma irradiation (kGy) on water activity after 72 hr of HM-4 and HQPM-1 maize genotypes. The error bars represent error at 5%.

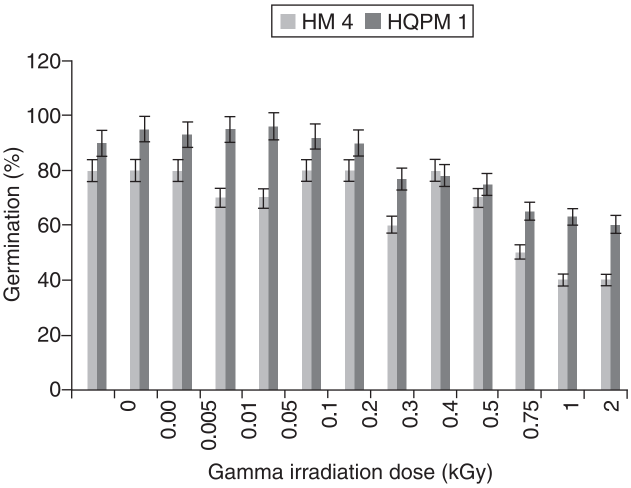

Fig 2 Effect of gamma irradiation (kGy) on germination per cent of HM-4 and HQPM-1 maize genotypes. The error bars represent error at 5%.

per cent for the HQPM 1 did not change significantly upto 0.2 kGy dose. However, germination per cent decrease at 0.3 kGy by 14.4% and the maximum reduction was recorded at 2.0 kGy by 33.3%. These comparisons were made with germination per cent of controlled (untreated seeds) (Fig 2). Delia Marcu *et al*. (2013) have reported that the germination per cent of maize genotypes reduces as the gamma irradiation dose increases. Seedling length of maize genotypes was influenced by the gamma irradiation doses. It could be said that the lower doses (<0.2 kGy) are significantly beneficial for seedling growth. The seedling length reduced on or beyond 0.2kGy dose significantly for both the type maize genotypes (Table 1). Analysis for the HM 4 maize genotypes revealed the increase in the seedling length by 9.1%, at 0.005, 31%, at 0.01, 31% at 0.1 and by 32% at 0.1 kGy. However, seedling length reduced compare to control (0.00KGy) at 1.0 and at 2.0 kGy by 77.4% and by 81.8% respectively. Similarly the assessment for the seedling length for HQPM 1 stated that it increased at 0.005, 0.01, and 0.05 and at 0.1 kGy by 15%, 46.3%, 51% and 24% respectively. The seedling length of HQPM 1 mostly

Table 1 The physiological parameters of plant seedlings for average of three replications

	HM 4				HQPM 1			
Irradiation dose (kGy)	Seedling length (cm)	Dry weight (g)/ seedling	Seedling vigor index I	Alpha amylase activity (µmole/ g/minute)	Seedling length (cm)	Dry weight/ seedling (g)	Seedling vigor index I	Alpha amylase activity (µmole/ g/minute)
00	10.4(±2.7)	0.164 (±0.06)	832 (±3.1)	11.02(±2.19)	22.1(±2.0)	0.115(±0.05)	1989(±2.09)	11.6(±2.9)
0.0025	10.5(±1.7)	0.199(0.028)	720(±4.0)	12.6(±2.56)	25.0(±2.9)	0.153±(±0.06)	2375(±2.19)	13.21(±3.0)
0.005	11.4(±3.6)	0.21(0.07)	912(±4.2)	12.1(±1.9)	32.3(±2.2)	0.153(±0.11)	3003(±3.5)	13.5(±2.9)
0.01	13.5(±3.3)	0.23(±0.015)	1012(±3.5)	13.1(±2.2)	33.2(±2.6)	0.205(±0.02)	3154(±3.7)	13.84(±2.0)
0.05	13.1(±1.3)	0.26(±0.01)	982(±1.99)	13.2(±199)	27.5(±2.6)	0.212(±0.1)	2640(±4.5)	13.1(±2.3)
0.1	13.6(±1.1)	0.203(±0.02)	1088(±1.5)	13.62(±2.3)	23.6(±3.1)	0.213(±0.03)	3171(±3.6)	13.55(±1.6)
0.2	10.3(±1.0)	0.2(±0.01)	824(±1.4)	11.9(±1.23)	13.5(±1.9)	0.233(±0.2)	1215(±2.3)	9.1(±3.1)
0.3	9.0(±0.8)	0.22(±0.02)	540(±1.1)	11.84(±2.9)	12.8(±1.3)	0.142(±0.3)	985(±2.1)	8.8(±1.61)
0.4	8.0(±1.2)	0.162(±0.05)	640(±1.5)	11.3(±3.1)	14.3(±1.9)	0.133(±0.01)	1115(±2.1)	8.11(±1.99)
0.5	4.0(±0.5)	0.178(±0.02	280(±0.8)	10.1(±185)	10.1(±3.1)	0.11(±0.02)	757(±1.9)	7.9(±1.95)
0.75	3.33(±0.3)	0.18 (±0.02)	199(±1.1)	8.2(±1.65)	4.5(±0.5)	0.122(±0.03)	292(±1.3)	7.6(±2.1)
1.0	2.5(±0.25)	0.131(±0.04)	100(±0.88)	7.6(±1.22)	4.2(±0.8)	0.122(±0.02)	265(±1.1)	7.7(±2.3)
2.0	3.0(±0.6)	0.130(±0.01)	120(±0.75)	7.9±(±1.12)	4.0(±05)	0.1(±0.02)	240(±1.0)	7.9(±1.85)

Data are the means of ten replicates± SD [Standard Deviation)

reduced by 82% at 2.0 kGy followed by 81, 80 and by 50% reduced at 1.0 kGy 0.75 kGy, 0.5 kGy respectively (Table 1). It may be attributed to reduced mitotic activity in tissues and reduced moisture content in seeds. These results are in line with Din *et al.* (2003), Kon *et al.* (2007) and Sharma and Rana (2007). One way ANOVA analysis for plant seedlings length revealed that the F values for the HM 4 and HQPM 1 are 214.74 and 201.5 respectively, which, are much greater than the corresponding critical values of $F_{critrical}$14.37 at $\alpha = 0.001$, since F values are greater than the critical values that is why results are significant at the level of 0.001% ($P<0.00$).

Data gathered for alpha amylase enzymatic activity for both the genotypes states that the alpha amylase activity increased in lower doses of gamma irradiation (< 0.2 kGy) and it reduced at 0.2 kGy dose or beyond significantly. The alpha amylase enzymatic activity for HM 4 maize genotype reduced by 16% at 1.0 kGy and by 13% at and 2.0 kGy (Table 1). Similarly the alpha amylase enzymatic activity was also assessed for the HQPM 1 maize genotype and it increased in the lower doses of gamma irradiation. The alpha amylase activity reduced the most at 2.0 kGy by 17.2%. These results were compared with the alpha amylase activity of control seeds. Change in the alpha amylase enzymatic activity corresponds to the change in the seedling length of both the genotypes (Table 1). Stanley *et al.* (2005) reported that complex carbohydrates broken down with the help of α-Amylase enzyme activity.

Dry weights of seedlings data were estimated for HM 4 as well as HQPM 1 maize genotype. And it is found that the seedling dry weight of HM 4 increased in the lower doses of gamma irradiation. At the lower dose the seedling dry weight was largest by 40% for 0.005 kGy dose. However the seedling weight mostly reduced at 2.0 kGy dose by 21% (Fig 3). Likewise the data were assessed for the HQPM 1 maize genotype and it is observed that the dry weight of seedling showed similar trend like HM 4 and it is larger at 0.2 kGy by 51% and at higher dose the seedling weight reduced by 13.6% at 2.0 kGy. These comparisons were made with control (zero dose) seeds (Fig 3).

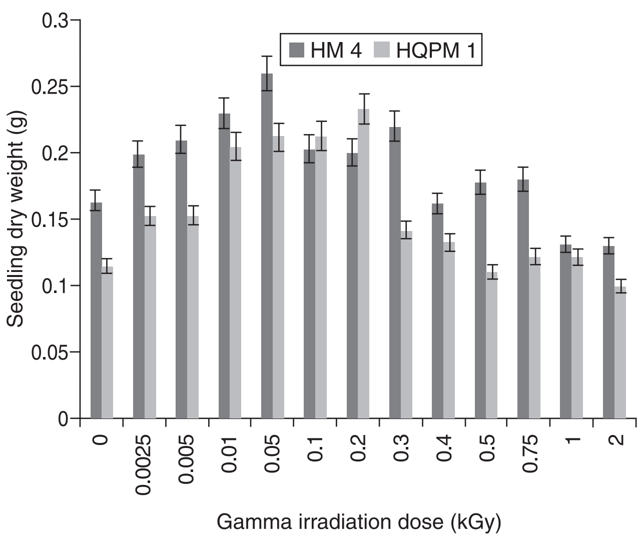

Fig 3 Effect of gamma irradiation (kGy) on seedling dry weight of HM-4 and HQPM-1 maize genotype. The error bars represent error at 5%.

An assessment was done for the root to shoot ratio. From analysis of data it is evident that the root and shoot ration increased in the lower doses (<0.2 kGy) and it has decreased on 0.2 kGy and beyond for both the genotypes (Fig 4).

The seed quality can influence many aspects of performance (e.g. total emergence and rate of emergence). The seedling vigor index I was determined and analyzed (Table1). One way ANOVA analysis of data for seedling vigor index I revealed that the F value for seed vigor index for HM 4 and HQPM 1 maize genotypes were 179.17 and 24.2 respectively. Statistically it is highly significant at level

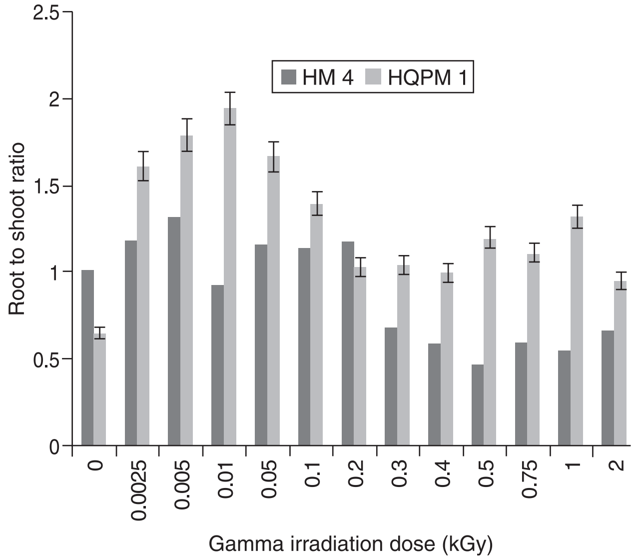

Fig 4 Effect of gamma irradiation (kGy) on root and shoot ratio of HM-4 and HQPM-1 maize genotype. The error bars represent error at 5%.

of 0.001% (P<0.00). It could be attributed to the excitation of molecules in the lower dosed of gamma irradiation and change in mitotic activity. Aparna *et al.* (2013) reported that seedling vigor index expressed higher reduction at the higher doses of gamma irradiation as compared to the non irradiated control seeds.

Correlation matrix is prepared with 12 degrees of freedom for HM 4 maize as well as HQPM 1 maize genotype. Analysis for the HM 4 maize genotype reveals that a positive linear relation exists between seedling dry weight per seedling and seedling length. The correlation coefficient is 0.57and it is significant at the level of 5%. Similarly the seedling length and seedling vigor index are positively linear related and the correlation coefficient is 0.969 and this relation is significant at the level of 0.1%. Alpha amylase activity and seedling length of HM 4 maize genotype are positively linearly related with correlation coefficient 0.95 and it is significant at level of 0.1%. Seedling vigor and the dry weight per seedling are positively linearly related and the correlation coefficient is 0.641 and it is significant at the level of 2.5% (Table 2). Alpha amylase activity and the dry weight per seedling are linearly related. The correlation coefficient is 0.586 and it is significant at the level of 2.5% (Table 2). Seedling vigor index and alpha amylase activity are positively linear related with 0.975 correlation coefficient and it is significant at the level of 0.1%(Table 2).

Correlation matrix for HQPM 1 reveals that the seedling length and dry weight per plant are positively correlated and the correlation coefficient is 0.832, which is significant at the level of 0.1% (P<000). Similarly the seedling vigor index is highly positively linear related with the seedling length and the correlation coefficient is 0.988 and it's significant at the level of 0.1%. Correlation between seedling vigor index and dry weight per seedling has been worked out and it could be said that it is highly positively correlated. The correlation coefficient for these parameters is 0.792 and it is significant at the level of 1%. Alpha amylase activity of seeds after irradiation is positively linear related with seedling vigor and the correlation coefficient is 0.954 (Table 2) and it is significant at the level of 0.1% (P<0.000). Likewise amylase activity and dry weight per seedling are positively linearly related. The correlation coefficient is 0.955 and it is significant at the level of 0.1%. These results confirm the earlier findings of Singh *et al*. (2009) of gamma irradiation onto the seeds of cereal crops.

In conclusion it could be said that gamma irradiation did not adversely affects the germination per cent at the lower doses (<0.3 kGy). However gamma irradiation to the seeds at higher doses (>0.2 kGy) prohibits the germination per cent significantly. Gamma irradiation to the seeds influences biochemical composition of seeds and it follows

Table 2 Correlation matrix of different physiological parameters in two maize genotypes (linear corr. coefficient)

	HM 4				HQPM 1			
	Seedling length (cm)	Dry weight (g)/ seedling	Seedling vigour index I	Alpha amylase activity(µmole/ g/minute)	Seedling length (cm)	Dry weight (g)/ seedling	Seedling vigor index	Alpha amylase activity (µmole/ g/minute)
Seedling length (cm)	1				1			
Dry weight (g)/seedling	0.5708	1			0.8322	1		
Seedling vigour index	0.9691	0.6417	1		0.9887	0.7923	1	
Alpha amylase activity (µmole/g/ minute)	0.9501	0.5868	0.9765	1	0.9548	0.8312	0.9552	1

the same trend as the germination per cent of both the maize genotypes. Seedling physiological parameters such as its length, dry weight and seedling vigor improve at the lower doses of gamma irradiation (<0.2 kGy) for both the maize genotypes but at higher doses seedling physiological parameters decreases on or beyond 0.2 kGy gamma irradiation dose.

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