Effect of Gamma Rays on Germination and Photosynthetic Pigments of Maize (*Zea Mays* L.) Inbreds

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ABSTRACT

This investigation was carried out to determine the effects of gamma radiation on germination and photosynthetic pigments of two maize inbred lines (RML-17 and RML-32). The pure dry seeds were irradiated with variable dosages (200, 250, 300 and 350 Gy) at the rate of 65cGy/min from ^{60}Co source. The results showed that there was a significant decreasing effect of the gamma rays on the final germination percentage (FGP) but the rate of germination was not significantly affected by radiation dosages. However, a decreasing trend was observed in general for the germination rate. The higher dose (350Gy) of gamma rays was found to have the maximum inhibitory effect on FGP for both inbreeds (31.2% for line RML-17 and 33.3% for RMl-32).The inhibitory effect of gamma rays was seen for the photosynthetic pigments especially, the chlorophyll-a [minimum at 350 Gy(6.25mg/gm Fw) for Rml-32].The non-irradiated samples in both inbreed exhibited higher chlorophyll-a content(11.056gmg/gm FW for RMl-17 and 11.74mg/gm Fw for RML-

32). The effect of gamma rays on chlorophyll-b content was no significant but a decresing effect was seen in higher radiation dosages. The total chlorophyll content was found significantly affected by dosage for line RMl-32,it was found maximum (21.25gm/mg Fw) for non-irradiated sample with the minimum total chlorophyll content occurring at 350Gy(13.47mg/gm FW).Furthermore, the concentration of chlorophyll-a was higher than chlorophyll-b in both irradiated and non-irradiated plants except at 350Gy for line RML-32 where chlorophyll-b7(7.21mg/gm FW) was found maximum compared to chlorophyll-a(6.25mg/gm FW). The overall effect of the gamma rays was inhibitory for all the traits under the study.

Key words: Maize, Gamma rays, Final germination percentage, Photosynthetic pigment

INTRODUCTION

Maize (*Zea mays* L.) is globally important cereal crop, mostly being used for food, feed and industrial purpose. The developing countries cover about 73% of the 153 million ha of total maize cultivated world wide, with annual increment in production estimated to be 6% (Prasanna, 2011). In spite of large coverage of cultivable area, the average productivity is not satisfactory. The demand for Maize consumption is expected to exceed that of rice and wheat by year 2020 (Prasanna and Hoisington, 2003) due to its variable use and it is predicted to have highest production in developing countries by 2050 (Prasanna, 2011).

Various approaches are carried out to increase the production of maize including nuclear techniques. Unlike conventional breeding programs, nuclear techniques focus on using physical mutagens to improve the traits in a plant. Among various physical mutagens, ionizing radiation (gamma rays) is used as major tool for nuclear breeding approach (Peri *et al.*, 2011). Gamma rays belong to the ionizing radiation which interact with molecules in the cells and produce the free radicals which have the potential to damage or modify cell characteristics (Minisi *et al.*, 2013). Gamma irradiation is widely used as modification agent for improving genetic diversity in agriculture due to its high penetration ability as compared to other ionizing radiations (Akshatha *et al.*, 2013). Its exploitation in agriculture is limited due to uncertainty in the dose of irradiation which varies for different crops and application (Peri *et al.*, 2011).

Previous studies have shown that application of Gamma rays has reduced germination percentage and plant survivals due to gathering of phenolic compounds (Minisi *et al.*, 2013 and Aly, 2010). The seed embryo subjected to higher level of radiation results in chromosomal injury responsible for less germination and survival (Akshatha *et al.*, 2013).While higher dosages of Gamma ray are known to be detrimental to plant DNA due to their damaging effect, different plants may exhibit different level of tolerance. The physiological effect of gamma radiation is due to the formation of free radicals by the hydrolysis of water, which may result in the modulation of an antioxidative system, accumulation of phenolic compounds and chlorophyll pigments (Kovacs and Keresztes, 2002; Kim *et al.*,2004; Wi *et al.*, 2007 and Ashraf, 2009).However, low dosages of Gamma radiation may be helpful in improving the enzymatic activation of young embryo, stimulating the rate of cell division and enhancing the production of plant metabolites for proper physiological development of the plant (Moussa, 2011).

MATERIAL AND METHODS

2.1 Procurement of seeds and irradiation

The dry seeds of two maize inbreed RML-17 and RML-32 which was procured from National Maize Research Programme (NMRP), Rampur, Chitwan, Nepal were taken as the material for irradiation. Each sample of 120 seeds/plate was taken in 8 plastic petri plates arranged in a single layer and irradiated with different dosages of gamma rays (200Gy, 250Gy, 300Gy and 350 Gy) at dose rate of 65cGy/min at room temperature (25 ± 1 °C) from Co^{60} gamma irradiator (Theraron Elite 100).the lab portion of the experiment was conducted at the Department of Radiation Oncology(B.P. Koirala Memorial Cancer Hospital ,Chitwan) and the field portion was conducted at a research field site located at NMRP, Rampur, Chitwan using a Randomize complete block design(RCBD) with three replicates during the time period from February –June 2013.

of coleoptiles was used as the index of germination. The germination percentage was calculated using the following formula:

(FGP) = (Number of germinated seeds after n days/ Total number of seeds) X 100

Germination rate was calculated according to the following formula (Hezagi and Hamideldin, 2009):

2.2 Field observation for final germination percentage and germination rate

Seed emergence was recorded after 7, 10 and 15 days of sowing. The emergence

$$\text{Germination rate} = \frac{(G1\ X\ N1) + (G2\ X\ N2) + \cdots \ldots\ldots\ldots\ldots (Gn\ XNn)}{G1 + G2 + \cdots \ldots\ldots\ldots\ldots\ldots\ldots Gn}$$

Where: G =number of seeds germinated after n days

2.3 Extraction of photosynthetic pigments

Leaves from developing plants were collected at three developmental stages: pre-flowering (55 day after sowing, DAS), flowering (65DAS) and post- flowering (90 DAS), to analyze the effects of the gamma irradiation on photosynthetic pigment contents. For different biochemical estimation the irradiated and non-irradiated plantlets were frozen in liquid nitrogen, ground to a powder with a mortar and pestle under chilled condition and kept in a freezer (-25 $^{\circ}$C) for further analyses. Lyophilized leaf powder were homogenized in 80% acetone (1gm powder/15ml acetone) and centrifuged at 10,000×g for 10 min. The supernatant was taken in 3ml cuvette and subjected to spectrophotometer (Genesys 10S Series, Thermo scientific) for the determination of chlorophyll-a and b at 646 and 663 nm, respectively. Chlorophyll-a (Ca) and chlorophyll-b (Cb) content were determined according to the following equation and expressed in milligram per gram fresh weight of plant material (Kiong, 2008):

Chlorophyll-a, Ca = 12.25 (OD663nm) – 2.79 (OD646nm),

Chlorophyll-b, C b = 21.50 (OD646nm) – 5.10 (OD663nm)

Total chlorophyll, Ca + C b = 7.15 (OD663nm) + 18.71 (OD646nm)

Where OD=optical density at given wave length in nanometer (nm)

2.4 Statistical analysis

Statistical analysis were conducted using R 3.0.3(R Core Team,2013) and the agricolae v1.1-8 package(de Mendiburu, 2014).Two-away Analysis of and Tukey's test for comparisons of means(Steel *et al*.,1997)was conducted to determine the variety and dose which showed significant differences. Regression curves were fit using the Microsoft Excel-2007.

RESULTS AND DISCUSSION

3.1 Effect of gamma rays on final germination percentage (FGP) and germination rate of RMl-17 and RML-32

The final germination percentage showed significant interaction with dose of irradiation. The dose-dependent decrease in final germination percentage was seen in both inbred lines examined in the study (Table1). The maximum FGP was found for the non-irradiated samples (84.7% for RML-17 and 84.66% for RML-32) for both the lines .While the minimum germination percentage (31.28% for RML-17 and33.333% for RML-32) was recorded at the dose of 350 Gy (Table 1).

The linear regression line showed the negative slope for the Line RMl-17 with

76.8 % of variation in final germination percentage being explained by dosages. Similar inhibitory effect of radiation was seen for the line RMl-32, where the negative slope (-0.13) explained the dose effect 78.1 % in decreasing the final germination percentage (Figure1). A non-significant effect was seen for the germination rate due to the exposure of the seeds at various dosages of radiation .The non-significant effect was accounted for both inbreds. However, in general there was the negative or inhibition effect on the germination rate as radiation dose was increased from 0 to 350 Gy. The inhibitory effect, revealed by the regression analysis, tells us that there was a strong relationship(R^2=0.711 and 0.548 for RML-17 and RML-32 respectively) between the dose and the germination rate for the inbreeds .

Table 1: Variation in final germination percentage and germination rate due to various dosages of gamma rays.

RML-17		
Dose	Germination percentage	Germination rate
0Gy	84.71667 [a]	13.987667
200Gy	48.33300 [bc]	8.988333
250Gy	38.14333 [c]	6.749667
300Gy	31.94400 [c]	5.873500
350Gy	31.28000 [c]	14.620400
RML-32		
0Gy	84.66333 [a]	14.197667
200Gy	70.83367 [ab]	11.986333
250Gy	55.55500 [bc]	11.656000
300Gy	47.21100 [bc]	8.229000
350Gy	33.33300 [c]	5.874833
F-test	***	ns
$HSD_{(0.05)}$	27.50	15.84

CV%	18.08	53.64

HSD: Honestly significant difference, CV: Coefficient of variation, Gy=Grey, Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05, ns :non-significant, Means with same letter are not significantly different

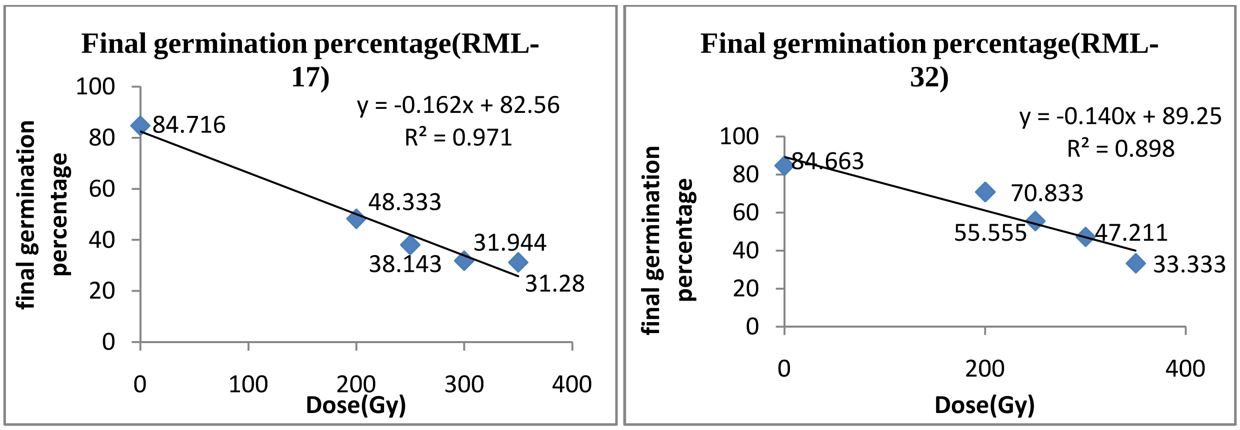

Figure1: Effect of gamma rays on Final germination percentage (FGP) for line RML-17 and RMl-32.

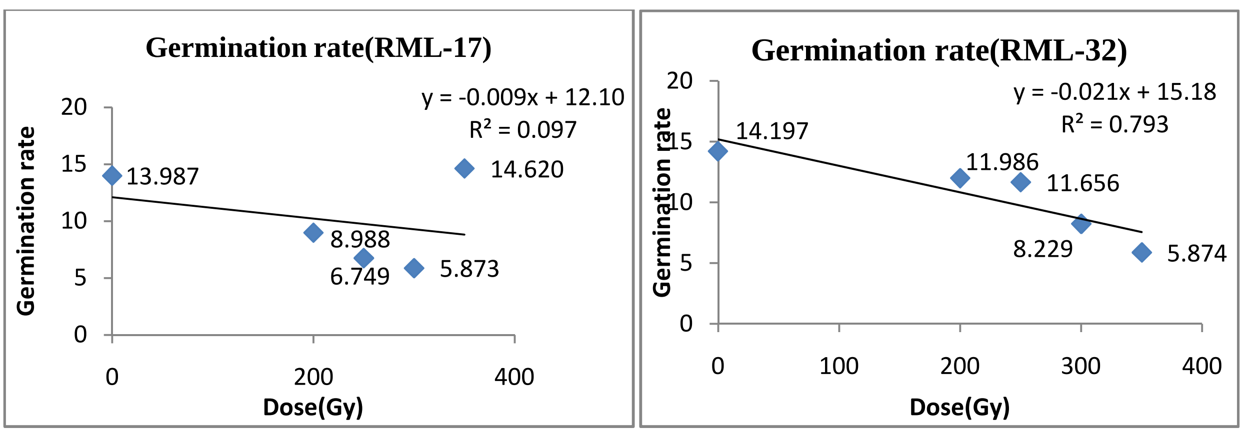

Figure 2: Effect of gamma rays on Germination rate (GR) of RMl-17 and RML-32

The reason behind the decrease in the final germination percentage could be the failure in the proper development of the plumule or the radicle in the course of the germination of the seeds (Basi *et al*., 2005). Raj *et al*. (1972) also reported a decrease in growth of the plumule and the radicle of rice with increases in irradiation dosage. These results are in consistent with the study by Kiong *et al*. (2008), who found that radiation increases plant sensitivity to gamma rays. They attributed this finding to a reduction in the amount of endogenous growth regulators, especially the cytokines, as a result of breakdown, or lack of synthesis, due to radiation. Similarly, Hameed *et al*., 2008 found that final germination percentage decreased significantly after higher irradiation dosages ranging from 350-500Gy. Maximum decrease in germination percentage was observed after 500Gy dosages. A similar observation was reported by Jan *et al*. (2011) that irradiation with lower dosages of gamma rays significantly improved vegetative traits while higher dosages proved depressing for same parameters.

3.2 Effect of gamma rays on photosynthetic pigments

The dose-dependent significant difference was found in chlorophyll-a content for both inbred lines. The maximum chlorophyll-a content occurred in non-irradiated samples(11.056mg/gm FW for RMl-17 and 11.741mg/gm FW for RML-32) while the application of gamma rays decreased the chlorophyll-a content .In context of RML-17 different gamma rays dosages(200Gy, 250Gy, 300Gy and 350Gy) on chlorophyll-a content are significant at par. The dose-dependent decreasing trend was found in line RML-32 for chlorophyll-a content. The mean comparison for various dosages revealed that there was significant inhibition effect of radiation with minimum (6.25 mg/gm FW) occurring at the dose of 350Gy. Chlorophyll-a content at a dose of 350 Gy was significantly different from any other dosages (Table 2).

On contrary, the amount of chlorophyll-b content did not vary significantly with the increasing dosages of radiation. Chlorophyll-a content was found to be higher than chlorophyll-b content in both lines for all treatments including the control. However, total chlorophyll content varied significantly with increasing dosages of radiation. In general the decreasing effect was observed for all dosages of radiation. In the case of RML-17, the decreasing effect was significant at par. The mean comparison between variable and treatment showed significant differences with decreasing effect of radiation for line RML-32.The none irradiated samples exhibited maximum total chlorophyll content (21.25mg/gm FW). Minimum total chlorophyll content was found at dose of 350Gy (13.47mg/gm FW) for RML-32(Table2). The linear regression line for total chlorophyll showed the negative slope for both inbreeds with 93.6% and 71.1%of variation in final germination percentage being explained by dosages for RML-17 and RML-32 respectively (Figure5).

Table 2: Effect of gamma rays on photosynthetic pigments (mg/gm FW) of line RML-17 and RMl-32

Dose	Chlorophyll-a	Chlorophyll-b	Total
RML-17			
0Gy	11.056543 [a]	8.848320	18.62618 [ab]
200Gy	9.777856 [ab]	6.596437	17.65298 [ab]
250Gy	9.876380 [ab]	7.103488	16.69179 [ab]
300Gy	9.665050 [ab]	5.873500	16.76854 [ab]
350Gy	9.998747 [ab]	6.419507	16.41825 [ab]
RML-32			
0Gy	11.741833 [a]	9.508494	21.25033 [a]
200Gy	10.940427 [a]	6.154808	17.09523 [ab]
250Gy	10.995534 [a]	7.358851	18.35439 [ab]
300Gy	9.749410 [ab]	8.244902	17.99431 [ab]
350Gy	6.255590 [b]	7.219100	13.47469 [b]
F-test	*	ns	***
$HSD_{(0.05)}$	4.64	5.59	6.05
CV%	16.04	26.06	12.01

HSD: Honestly significant difference, CV: Coefficient of variation, Gy=Grey, FW : Fresh weight Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’, Means with same letter are not significantly different

The regression line showed steep slope (-0.003 for chlorophyll-a and -0.007 for chlorophyll-b indicating a sharp decrease in RML-17. It explained that contribution of dosage for reducing chlorophyll-a content was 72.4% while it was 83.3% for chlorophyll-b content (Figure3).The trend was similar for line RMl-32 as well. The negative regression for line RML-32 suggested the strong cause for decrease in constituent chlorophyll pigments due to administration of radiation. It was 55.8 % for chlorophyll-a and 34.1% for chlorophyll-b (Figure 4).

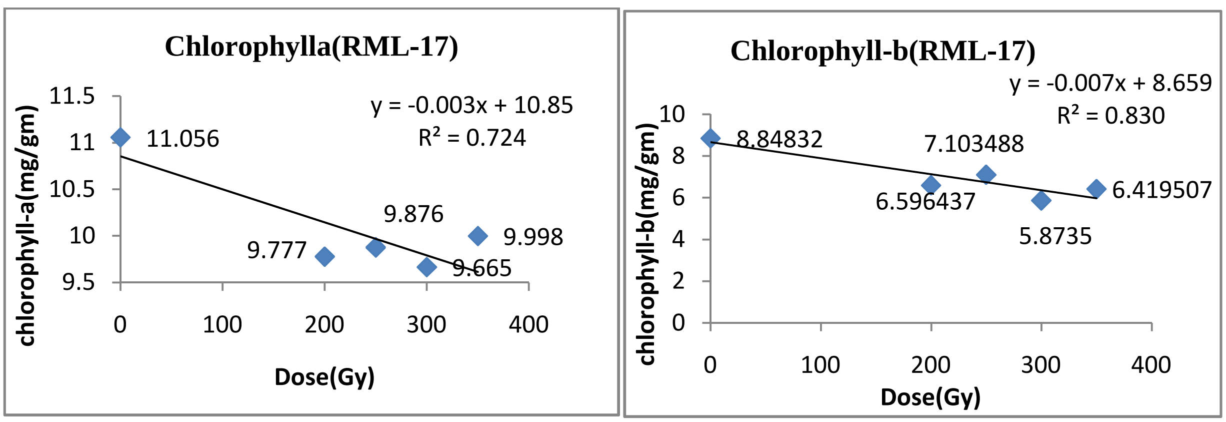

Figure 3: Effect of gamma rays on chlorophyll-a and chlorophyll-b content in line RML-17

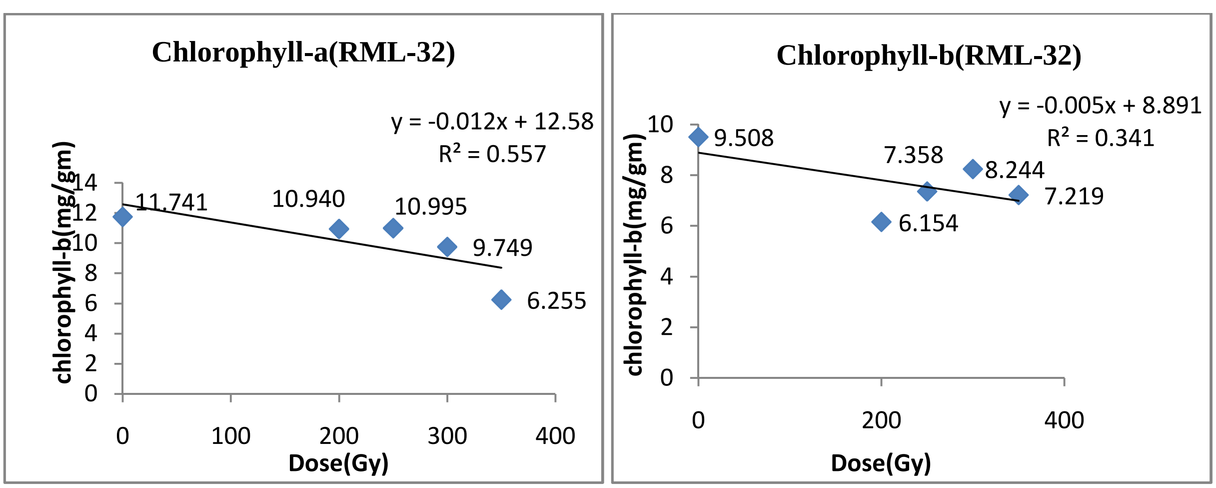

Figure 4: Effect of gamma rays on chlorophyll-a and chlorophyll-b content of line RML-32

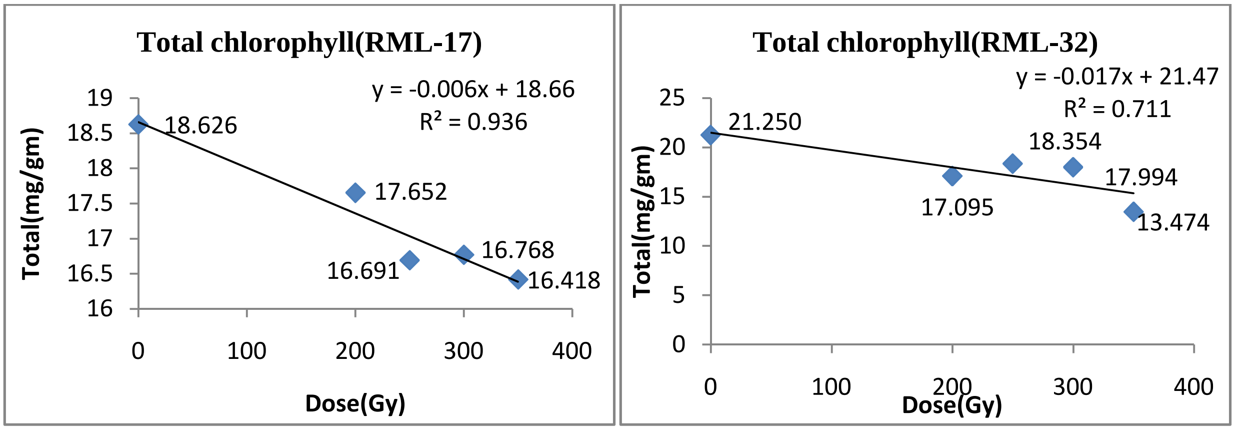

Figure 5: Effect of gamma rays on chlorophyll-a and chlorophyll-b content of line RML-17 and RMl-32

In this present study the chlorophyll content showed a regular decrement from non- irradiated plants to irradiated plants .These result are in accordance with the findings of Ling *et al.* (2008).Similar results were found in case of *Cullen corylifolium* for total chlorophyll content at various stages of plant growth by Jan *et al.* (2013).Furthermore, a decrease in higher dosages of gamma rays (20kGy) was found for chlorophyll-a and chlorophyll-b content in same research.

Similarly, in a study examining lettuce (*Lactuca sativa* var. capitata) dry seeds exposed to radiation dosages ranging from 2-70 Gy and findings indicated that seeds irradiated with dosages ranging from 2-30 Gy enhanced the photosynthetic pigments (Chl-a, Chl-b, Car) content, while higher dose (70 Gy) resulted in declines in the assimilatory pigments (Marcu *et al.*, 2013b).

CONCLUSIONS

Our results presented above are concerned with persistence of changes prevalent from seed to seed cycle, where the inhibition in germination percentage and photosynthetic pigments were more prevalent over the higher dosages around 300 to 350Gy. The morphological and biochemical characteristics of plants have been reported to be effected by gamma rays .Hence the gamma rays can be utilized as the modifying agents for certain plant characteristics at appropriate dosages which may provide a stimulatory effect.

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