Germination Viability of Maize M1 Seeds (*Zea mays* L.) after Gamma Ray Irradiation

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Abstract: A series of researches was carried out to obtain mutant of maize induced by gamma rays irradiation. This initial report was from a study confirming the best dosage of irradiation at 50% lethal dosage (LD_{50}) conducted at the Laboratory of Seed Science and Technology, Faculty of Agriculture, Hasanuddin University, Makassar from March to June 2012. The research was set up using split plot design of the Randomized Complete Block design. Data were analyzed using analysis of variance. Results showed that: Gamma ray dosage affected maize seed viability. The higher gamma ray dosage used resulted inmore inhibiton of maize seed germination. The best LD_{50} irradiation to induce putative mutation (M1) in maize seeds tested ranged from 100-200 Gy. The genotype Lamuru maize irradiated at 100 Gy were superior and were more viable with a percentage germination of 87.22% and vigor index of 4.48.

Keywords: Maize; gamma ray irradiation; seed viability

1. Introduction

The demand of maize as food or feed industry purposes tend to increase gradually. This has become the reason to develop maize which could be planted in marginal land such as on the dry and saline land. Dry land is land that is not optimally utilized as a result of water stress that limit plant growth in the field, whereas saline land was areas of land affected by sea water intrusion which contains about 3% NaCl, leading to salinization and resulted plants experiencing salt stresses, which would pose a serious threat to agricultural productivity (Munns, 2006). Planting of maize on dryland and or saline land required varieties suitable for planting in marginal land and able to adapt well to the stressful environment while still produces high production of maize. According to Guedev (2002), dominant factor causing low productivity of food crops were low application of cultivation technology in the field, land fertility rate continues to decline, and the exploration of new genetic potential was not optimal.

Utilization of the genetic potential to improve crop varieties through plant breeding could be done, for example by induction of mutations (Witjaksono, 2003). Induction of mutations by gamma irradiation has been used in the development of high yielding variety of crops resulting in greater phenotypic diversity. Plant mutation was expected to increase genetic diversity in breeding plant with improved resistance to abiotic stresses. Wide genetic diversity could be used as the basis for selection of plants which would provide greater possibilities and make it easier for plant breeders to make selections based on genotype breeding to obtain the best genotypes. The selective variability of genotypes for seedling tolerance to stress factors is a very important indicator for crop improvement (Turan *et al*., 2010).

Induction of mutations by gamma ray irradiation has been used in the development of high yielding varieties of carnation plants (Aisyah *et al*., 2009), and plant mutations in rice, soy and chilli have been carried out but still lacking in maize. Mutations induction in maize seeds allowed more genetic diversity to be obtained. To obtain solid mutants, irradiation could be performed on callus, somatic embryos, suspension cells or protoplasts or seeds Hemon (2009). Several irradiation dosages were used to induce the formation of diversity determine the success of the mutant plants.

There was no significant effect of irradiation dose to produce mutants. It happened very randomly. Although the level of gamma irradiation dose was given to the same plant, the effects may not be the same. Reproducing the same protocol on different type of plants would give even more random mutants. The effectiveness of irradiation must be first studied to determine the optimum dose. Doses that were too low may reduce the number of mutants formed while doses too high would kill the plant material used or lead to sterility. Proper plant mutations induction with optimum gamma irradiation dose was expected to increase the genetic diversity of maize.

2. Materials and Methods

2.1 Materials

Materials used in this study consisted of filter paper, water, and maize seed. Maize seeds (Bisma, Lamuru and Sukmaraga genotype) were irradiated at the Center for Application of Isotopes and Radiation Technology (PATIR - BATAN), Pasar Jumat, Jakarta, as per required of irradiation dose treatments (0, 100, 200, 300, 400, 500 Gy) producing 18 mutants (M1).

2.2 Methods

The experiment was to test several doses of irradiation on maize varieties tested, using a split plot design of randomized complete block design. The first factor was the varieties as main plots consisting of three levels, such as: Bisma, Lamuru, and Sukmaraga. The second factor was the sub-plot i.e. irradiation dose consisting of six levels (0, 100, 200, 300, 400 and 500 Gy). Combination of treatment produced 18 units, each was repeated three times representing a block.

The germination test was performed on a paper germination test method. Germination test conducted were carried out by placing 100 seeds on two pieces of scrap filter paper on a plastic plate to determine germination rate and seed vigor.

2.3 Data Analysis

Data analyses were carried out using the SPSS var. 18 software and Microsoft Excel. Differences between means of value were determined by Least Significant Different (LSD) test at probability level of 5%.

3. Results and Discussion

Seed was a candidate of crop plants containing genetic material. Seed germination was the beginning of the life cycle of plants, and the emergence of seedlings was essential for the formation of plant populations. Plants were generally more sensitive at germination and seedling stage than the adult stage (Khan and Gulzar in Tahir, 2009). Observations on all the parameters tested showed that each M1 seeds produced have different responses. Varieties and or dose of irradiation significantly affected the vigor index, germination percentage, fresh weight and dry weight of maize sprouts.

The LSD (Table 1) showed that the Lamuru genoptype was more viable than other varieties as indicated by the high percentage of germination (87.22%), vigor index (4.48) as well as having fresh weight

Table 1. Vigour index, percentage of germination (%), fresh weight (g), and dry weight (g) maize sprout of several varities and dose of irradiation

Varieties	Vigor indeks	Germination percentage	Fresh weight	Dry weight
Bisma	4,42 ^{k}	80,56 ^{l}	0,70 ^{l}	0,18 ^{l}
Lamuru	4,48 ^{k}	87,22 ^{k}	0,87 ^{k}	0,23 ^{k}
Sukmaraga	3,62 ^{l}	87,22 ^{k}	0,83 ^{k}	0,20 ^{kl}
Irradiation (Gy)				
0	3,94 ^{k}	97,78 ^{k}	0,93 ^{k}	0,21 ^{k}
100	3,65 ^{k}	94,44 ^{k}	0,87 ^{kl}	0,20 ^{k}
200	3,94 ^{k}	87,78 ^{l}	0,81 ^{lm}	0,22 ^{k}
300	3,74 ^{k}	83,33 ^{lm}	0,75 ^{mn}	0,19 ^{k}
400	3,95 ^{k}	77,78 ^{m}	0,75 ^{mn}	0,21 ^{k}
500	3,82 ^{k}	68,89 ^{n}	0,69 ^{n}	0,20 ^{k}

Note: Values followed by the same letter in column (k, l, m, n) are not significantly different (LSD, $P<0.05$)

(0.87 g) and dry weight (0.23 g). Figure 1 and Table 1 showed a pattern that the higher the concentration of a given irradiation dose led to a decrease in the percentage of seed germination and seedling fresh weight in all the varieties tested. Given no irradiation dose has the highest value, while a dose of 500 Gy has the lowest value of all the parameters observed. This meant that the irradiation on the seeds could cause physiological and gene disorders that impacted in the seed and it could also affect seed viability. Irradiation dose of more than 300 Gy has led to a decrease of seed germination below 85%. Even at a dose of 500 Gy seed germination could only reached 68.89% nearing the lethal dosage LD_{50}, which is below the minimum standard requirements for quality seeds and the irradiaton was too high. Based on these data it was concluded that the appropriate dose of irradiation on corn seed tested should only range between 100 - 200 Gy. Shuman and Sihono (2010) reported that sorghum seeds have LD_{50} of 504 Gy.

Only a small amount of seeds irradiated at 400 and 500 Gy were germinated. Furthermore, germinated seed was very weak and abnormal, unable to grow due to the discontinued growth of radicle or plumule and died after 14 days. Herison *et al*. (2008) stated that the abnormality of plant death growing from irradiated seeds could be caused by several factors resulting in changes in both the level and structure of DNA, cells, and tissues caused by the formation of free radicals such as H^+ ions that are highly unstable making physiological processes in plants become abnormal and produced new genetic variations.

The amount of irradiation dose given on the seed caused physiological damage on the internal structure of embryo of the seed. This response was different between genotypes. Irradiation doses were used to induce the formation of genetic diversity in determining the success of the mutant plants. Soedjono (2003) stated that factors affecting the formation of mutants among others were

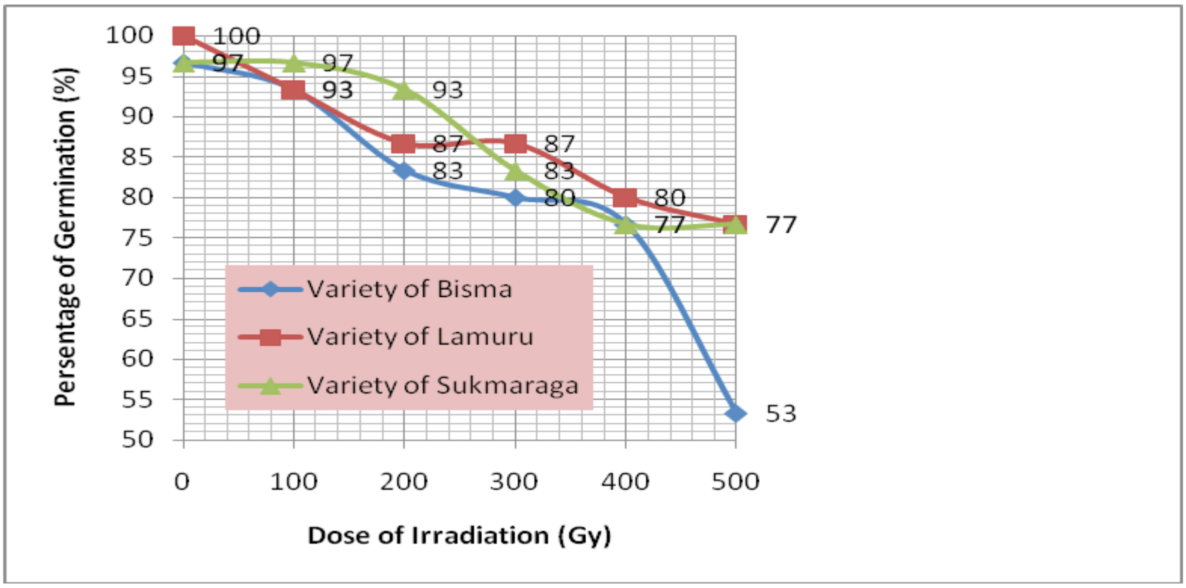

Figure 1. Germination percentage (%) on several varities of maize tested after irradiaton dose treatment (gray, Gy)

the amount of irradiation dose. Low doses are still able to maintain the vitality of buds and shoots, may extended the time to maturity of fruits and vegetables, increased levels of starch, protein and oil content in the seed corn, as well as having better resistant against pathogens and drought. However, in high doses could affect the metabolism severely so that germination can be disrupted and even killing the seed.

Interaction between varieties with irradiation dose affected (significantly and unsignificantly) to the length of plumule and radicle of maize sprouts. Table 2 showed that generally, the higher dose of irradiation on seed will suppress growth of plumule and radicle. The highest length of plumule developed was from M1 of Lamuru at 200 Gy (6.60 cm) while of Bisma at 500 Gy (1.57 cm) produces the lowest. The highest for radicle length of Bisma irradiated at 100 Gy (23.63 cm) and the lowest was found in the irradiated Bisma 500 Gy (3.73 cm).

The growth rate of the radicle seemed to be higher than plumule and based on the data collected it was found that the ratio of plumule to radicle length of Bisma was greater (0.42) than Lamuru (0.38) and Sukmaraga (0.34).

Gamma ray irradiations have different effects on plant seeds. Physiological disorder caused by high doses of the ability to influence the germination, growth and development including radicle plumula and thus affects the ratio of plumule to radicle length. Khodarahmpour *et al*. (2012) stated that the reduction in growth rate due to the decrease due to the expansion of cellular DNA damage.

The irradiation dose were also significant on the ratio of plumule to radicle length of Bisma and Lamuru and showed a quadratic pattern at $Y = -2E^{-06} X^2 + 0,001 X + 0,249$ (Figure 2). Germination test results showed that the dose of irradiation that should be used on maize seed from Bisma, and Lamuru variety were 100 - 200 Gy.

Table 2. Plumule length (cm) and radicle length (cm) of maize sprouts from several varities and irradiation dozes

Irradiation dozes (Gy)	Plumule Length			Radicle Length		
	Bisma	Lamuru	Sukmaraga	Bisma	Lamuru	Sukmaraga
0	8,13$^{a}_{k}$	6,57$^{b}_{k}$	6,25$^{b}_{k}$	25,90$^{a}_{k}$	23,30$^{b}_{k}$	22,93$^{b}_{k}$
100	6,17$^{a}_{l}$	5,25$^{a}_{l}$	5,42$^{a}_{kl}$	23,63$^{a}_{l}$	19,77$^{b}_{l}$	20,77$^{b}_{l}$
200	6,23$^{a}_{l}$	6,60$^{a}_{k}$	5,70$^{a}_{k}$	18,00$^{b}_{m}$	13,18$^{c}_{m}$	20,77$^{a}_{l}$
300	4,83$^{a}_{m}$	4,82$^{a}_{l}$	4,37$^{a}_{lm}$	7,00$^{b}_{o}$	12,07$^{a}_{m}$	10,98$^{a}_{m}$
400	3,60$^{a}_{n}$	3,50$^{a}_{m}$	3,08$^{a}_{n}$	11,90$^{a}_{n}$	7,62$^{b}_{n}$	8,03$^{b}_{n}$
500	1,57$^{b}_{o}$	1,67$^{b}_{n}$	3,47$^{a}_{mn}$	3,73$^{b}_{p}$	4,67$^{b}_{o}$	8,13$^{a}_{n}$

Note: Values followed by the same letter in row (a, b, c) or column (k, l, m, n, o, p) are not significantly different (LSD, $P<0.05$).

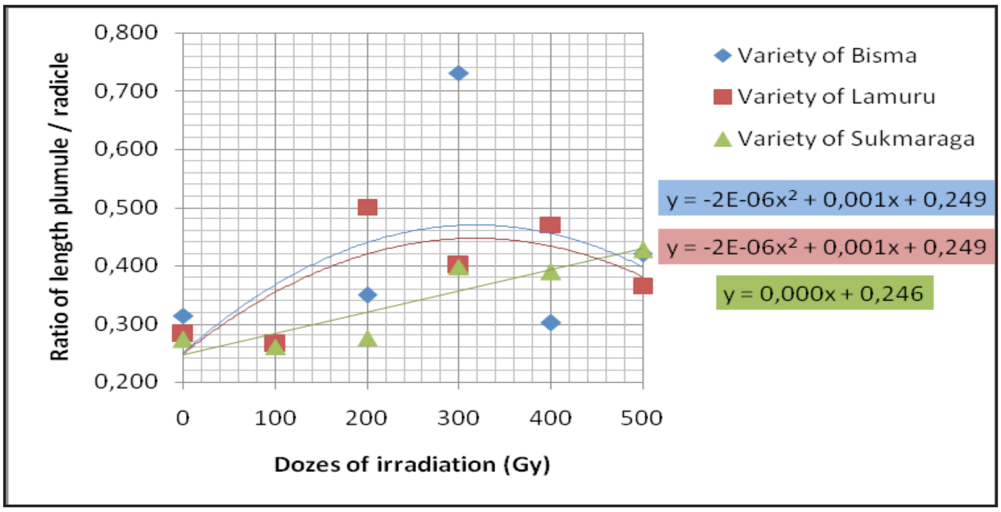

Figure 2. The ratio of length plumule / radicle of maize sprout from several varities and irradiation doze

4. Conclusion

Gamma ray dosages affected maize seed viability. The higher gamma ray dosage used, the more the maize seed germination inhibited. The best dose of irradiation to induce mutation (M1) in maize seeds ranged from 100 - 200 Gy. Lamuru irradiated at 100 Gy were more superior and gave more viability i.e. percentage of germination (87.22%) and vigor index (4.48).

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