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Mutagenic sensitivity analysis of gamma irradiations in Cowpea (*Vigna unguiculata* L. Walp)

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Abstract

A study was undertaken in Cowpea (*Vigna unguiculata* L. Walp.) seeds to assess the mutagenic sensitivity of physical mutagen. Germination percentage gradually decreased in general with increase in dose/concentration of mutagen as similar condition was found in case of plant height. Here, 9and 14 different gamma doses were used to study the LD_{50} and GR_{30}/GR_{50}, respectively. LD_{50} value was 590.03 Gy based on germination percentage. GR_{30} and GR_{50} values based on regression formula on shoot length were 179.50 Gy and 318.68 Gy, respectively. The doses in between the range of 318 Gy - 179 Gy doses were most appropriate to induce variation in cowpea.

Keywords GR_{30}, GR_{50}, LD_{50}, mutagen, regression

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is a leguminous species used as food, forage, and vegetable crop [1]. The grains are an excellent source of food and feed; a vital nutrient for healthy growth of both humans and livestock [2].

Genetic diversity in crops is the foundation for plant breeding programs. Most traditional crop upgrading programs depend on natural genetic variation existing amid germplasm pools [3]. Induced mutagenesis has the prospective to generate variation for genetic enhancement and breeding in a comparatively shorter time unlike natural mutation or controlled crosses of especially unrelated parents [4]. The Mutant Varieties Database (MVD) of FAO (Food and Agriculture Organisation of the United Nations) and the International Atomic Energy Agency (IAEA) retained a list of 2,252 crop cultivars established via artificial mutations [5]. These cultivars were released across 59 countries worldwide, mainly in the continental Asia (1,142 cultivars), Europe (847), and North America (160) [6]. Studies show that developed mutagenesis has effectively altered several plant traits such as disease resistance, seed shattering resistance, maturity, plant height, , size and quality of starch granules, malting quality, oil quality and quantity of cowpea [7].

In mutational breeding experiments, the plant materials are exposed to radioactive particles or chemical compounds which may change the genetic constitution of plants. The physical mutagens are comprised of ionized radiations viz., particulate (alpha radiation and beta radiation). In general, ionizing radiations such as X-rays and γ-rays are more preferred than other mutagens because of their ease of application, good penetration and

Table 1. Germination percentage analysis of gamma ray treated seeds

Doses (Gy)	Number of total seeds	Number of germinated seeds	Germination Percentage
0	31	30	96.77
50	22	21	95.46
100	15	11	73.33
150	13	10	76.92
200	26	22	84.62
300	14	10	71.43
500	29	20	68.97
600	18	12	66.67
700	31	21	67.74
800	27	3	11.11

Table 2. Growth reduction (GR_{50} and GR_{30}) analysis of gamma ray treated seeds

S No.	Doses (Gy)	Plant height
1	0	100.00
2	50	90.77
3	100	99.85
4	150	90.14
5	200	82.24
6	250	60.27
7	300	35.08
8	350	21.97
9	400	15.81
10	450	12.85

reproducibility, high mutation frequency and less disposal problems [8]. Mutation may be used to obtain superior mutant for different traits like high yield, drought tolerance, disease resistance, quality, color, taste etc. The efficiency of mutation breeding is dependent on the effectiveness with which useful variants can be recognized in M_2 or M_3 generation [9]. The first step in the mutation breeding selection process is to reduce the population of potential variants to a sufficiently small fraction to permit more detailed analysis and evaluation. In order to determine the optimal dose of gamma irradiation, subsequent growth rate is recorded from the treated population. The plantlet height is used to determine optimal dosage for mutation induction as growth reduction GR_{30} and GR_{50}. The determination of LD_{50} value helps to define exact mutation dose [10]. The plant sensitivity to irradiation varies according to species, cultivar and the plant's physiological conditions [11]. In focused with above content, here we determined the LD_{50} value and the GR_{50}/GR_{30} values of gamma irradiations in cowpea.

Methodology

Selection of materials

The physically pure quality seed material free from any type of pathogenic infection or insect infestation was selected for irradiation. Seed moisture content is very important because radiolysis of water play an important role during irradiation of seed. The seed moisture content should be kept between 12 to 14%. Here, we used good quality Cowpea seeds as per the above mentioned criteria.

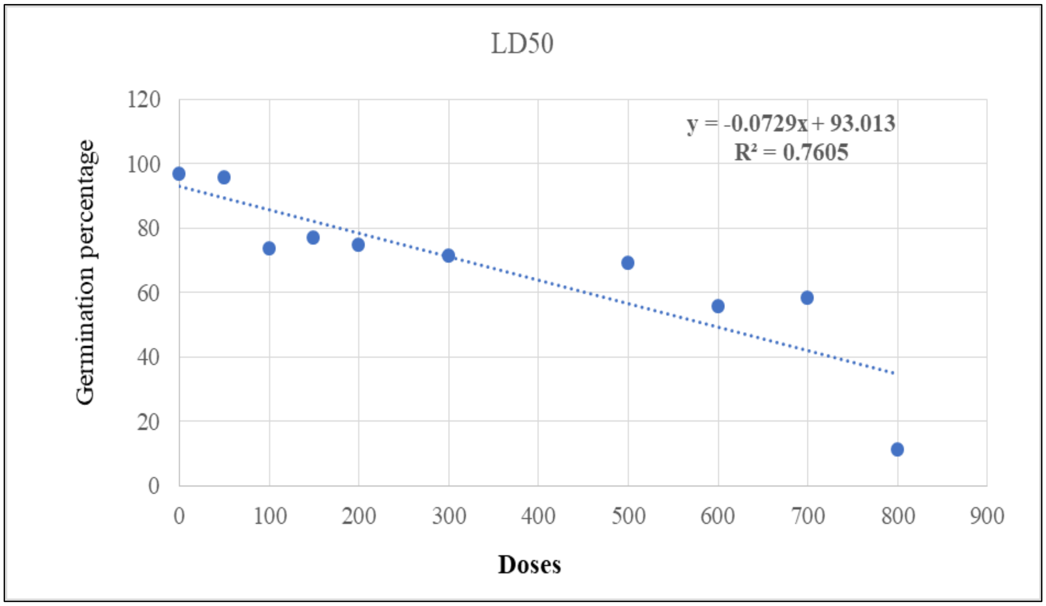

Figure 1. Graphical representation of germination percentage of gamma ray treated seeds
(N.B.: In Y axis germination percentage of seeds and X axis different doses of gamma radiation plotted)

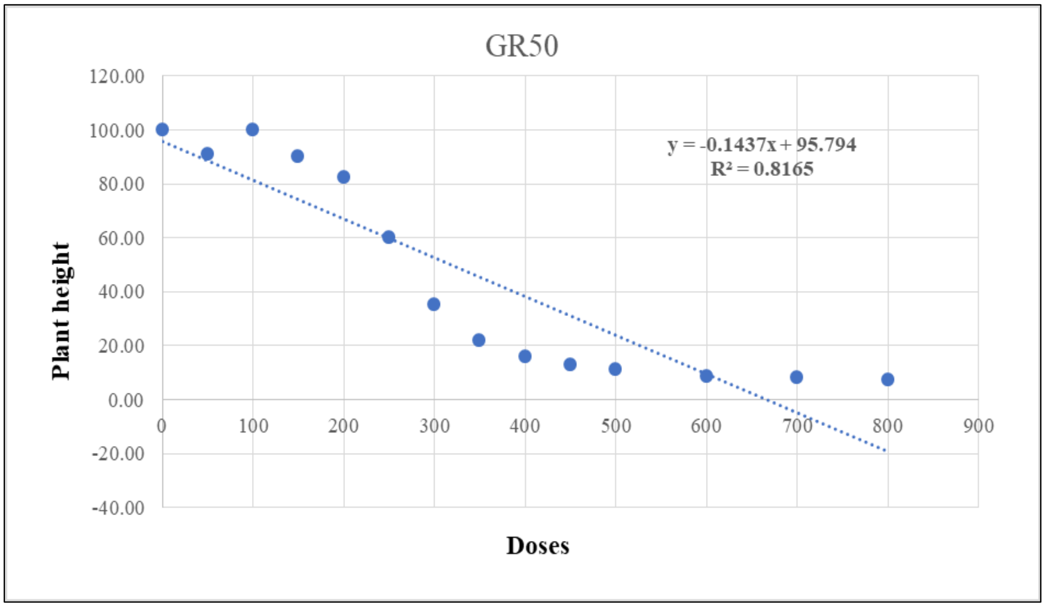

Figure 2. Graphical representation of GR_{50} of gamma ray treated seeds
(N.B.: In Y axis plant height and X axis different doses of gamma radiation plotted)

Source of Radiation

In the present experiment, seeds of cowpea (Vigna unguiculata L. Walp) were irradiated with different doses of gamma rays viz. 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, 400 Gy, 450 Gy 500 Gy, 600 Gy 700 Gy, 800 Gy along with control (0 Gy). Hence, there were a total of 14 treatments for determination of GR_{30} and GR_{50} value [12-14]. In the same way for LD_{50} analysis, seeds were irradiated with different doses of gamma rays viz. 50 Gy, 100 Gy, 150 Gy, 200 Gy, 300 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy along with control (0 Gy) [12-13, 15-16].

Rising of irradiated seed

The treated and control seeds were sown using Flat method. In this method, the seeds were sown in the plastic tray filled with a sterile soil and sand mixture. First of all, 14 rows were created in the sand-soil mixture. In each row, minimum 15 seeds were sown per treatment and covered with additional soil. Each row and tray was marked with given dose and specific number, respectively. Thereafter, water was sprinkled over the tray to provide sufficient moisture for seed germination and tray was finally kept in the germinator. For germination, percentage analysis data was recorded in seven days after sowing and the seedling height (shoot length) was recorded after ten days of sowing [12].

Results and Discussion

The median lethal dose (LD_{50}) and the median growth reduction (GR_{50}) are parameters utilized to establish the adequate irradiation dose to induce mutations in plant breeding programs. Mutation induction has the purpose of generating genetic variability, which may allow to identifying new desirable characteristics that are not found in nature. Several researchers agree that the highest probability to generate useful mutations for breeding programs occurs at doses where 50% of the irradiated individuals die [17]. Likewise, other researchers pointed out that another dose with a high probability of producing effective mutations, besides the LD_{50}, is where 50% of growth reduction (GR_{50}) occurs. Notably, both parameters (LD_{50} and GR_{50}) are based on the assumption that low doses of irradiation produce minimum impacts on the genome, which rarely generate phenotypic changes; whereas high doses may produce multiple impacts on the genome which consistently produce aberrations or negative changes. Therefore, the first step in a mutagenesis-based breeding process is to determine the LD_{50} and the GR_{50} [18].

In this experiment, germination percentage (Table 1) and plant height (Table 2) were gradually decreased as compare to control. LD_{50} value was 590.03 Gy (Figure 1) through regression-based formula on germination percentage, whereas GR_{30} and GR_{50} values based on regression formula on shoot length were 179.50 Gy and 318.68 Gy (Figure 2), respectively [17, 19-23].

On the basis of above data, we can interpret that for identification of desirable mutant LD_{50} (590.03 Gy) value is not a suitable criterion to decide doses of mutagen because survival of mutant is important than germination. In this condition for optimize doses of mutagen; growth reduction of 50 and 30 values will give better idea for finding useful mutant at specific mutagen rate. Hence, the doses range between 179.50 Gy (GR_{30}) and 318.68 Gy (GR_{50}) is ideal for induction of maximum desirable mutation in Cowpea.

Conclusion

As we know, GR_{50} value has the maximum chance to develop mutants. In this experiment, 318.58 Gy (GR_{50}) is the most acceptable dose for further cowpea mutation breeding programs. The findings may assist as reference doses for large-scale gamma irradiation of cowpea genotypes to induce genetic variation.

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