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Research Article

Effect of proton beam irradiation on survival and seedling growth parameters of Indian rice (*Oryza sativa* L.) variety 'Indira Barani Dhan 1'

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Abstract

Rice crop, a staple food for a majority of population across the world, holds a significant role to play in alleviating the global hunger problem. Enhancement of genetic diversity of rice will serve to overcome constraints in sustained and ecologically effective improvement in the production of quality rice, challenged by erratic patterns of global climate, changing pest dynamics, resource availability in proportion to growing population etc. Proton ion beam has sprung up as a promising novel mutagen in the mutation breeding of crop plants, by virtue of its higher LET values, causing higher frequency of mutations. Dose optimization is pre-requisite to obtain a range of phenotypic mutants, without drastic reduction in survival and growth subsequently. This study was undertaken to standardize proton beam irradiation dose rate post the evaluation of *in-vitro* germination, growth and survival of rice variety IBD -1, with doses ranging from 0-500Gy. Doses between 152.47Gy-253.53Gy were found suitable for mutagenesis, without drastically impairing growth and survival.

Key words
Proton beam, Linear Energy Transfer(LET), Lethal Dose(LD), Growth Reduction (GR), Dose optimization

Introduction

The rice (*Oryza sativa* L.) plant is an annual grass, grown under a wide range of climatic and geographical conditions in all five major continents. Among the major cereal crops, rice crop owns prominence in feeding more than 60% of world's population. Global Rice production statistics reveal production of 486.2 million metric tonnes milled rice in 2016-17 (https://www.statista.com/statistics/271972/world-husked-rice-production-volume-since-2008), with estimated world acreage of 161.1 million hectares (https://www.statista.com/statistics/271969/world-rice-acreage-since-2008).India, with a rice production volume of approximately 165.3 million metric tons, is ranked second in global rice production in 2016-17, with estimated harvest area of about 44.5 million hectares (https://www.statista.com/statistics/255937/leading-rice-producers-worldwide). With the burgeoning size of world population at an unprecedented rate, limited fertile land resources, climate resilience, emerging new races of pests and diseases and consumer preferences for quality attributes, it is imperative to increase crop diversity with better selection efficiency addressing the challenges of future rice production. In order to meet the dietary requirements of increasing population and challenges emerging due to the adverse impacts of climatic changes, genetic gains in the grain yield per hectare of major crops like rice, need to rise faster than the current rate (Challinor *et.al.*, 2014 and Ray *et.al.*,2012).

The genetic architecture of living organisms is influenced by mutations, in a confounding manner. Mutations form the foundation for selection, which ultimately is the driving force for progressive breeding among crop plants. This driving force may have a natural (spontaneous) origin or it may be artificially induced. Spontaneous mutationsare rather rare and random events and takes millions of years to evolve. Not only this gradual process can be greatly accelerated through artificial induction of mutation, but it also supports the maintenance of biodiversity. Germplasm resources act as a reservoir having tolerance to various emerging stresses which can be used in breeding programme to help introgression of traits in elite crop varieties. Conventional breeding is limited by tight linkages (genetic load) of undesirable traits and genetic differentiation at the expense of genetic diversity (Rauf *et. al.*, 2010). For rice breeders, it is a continuous task to find the new sources of genomic variation that can be directly used in rice breeding programs (Lee *et. al.*, 2015). Mutation breeding is an efficient technology in the hands of plant breeder which helps in breaking undesirable linkages and also results in genetic changes desirable for breeders in the background of an elite variety. In rice, more than 820 mutant varieties have been developed through mutation breeding (http://mvd.iaea.org/).

Radiobiological effects are known to vary with physical modifying factors such as linear energy transfer (LET), type of radiation, fractionated and protracted irradiation. Biological response towards various type of radiation depends largely on type of interaction and the way energy is deposited in the media (Bhat *et. al.,* 2015).In plant mutagenesis, the energy and penetration ability of the radiations are the two key technical parameters that affect the effectiveness of this physical mutagen. Ion beam forms novel yet powerful alternative physical mutagen with mutation spectrum different from other forms of mutagenic radiations. Ion beams, owing to their high LET, result in localized deposition of energy and clustered damage, contrary to gamma rays or X-rays, consequently leading to more double-strand breaks resulting in higher frequency of useful mutations (Magori *et.al.*, 2010). Efficient induction of novel mutants, without inducing undesirable traits, through the use of ion beams has been speculated on the basis of high mutation frequency, larger but limited number of DNA lesions and broad mutation spectrum (Tanaka *et.al.*, 2010).

Proton beam irradiation has recently earned an eminent role to play in mutation breeding. In this type of mutagenesis, positively charged ions, accelerated at a high speed (around 20–80% of the speed of light), are used to irradiate target cells (Oladosu *et.al.,* 2015). Energy of proton beam lies up to several GeV while the penetration lies up to few cm. In spite of its potential as an impressive physical mutagen, there have been scanty reports on utilization of proton beam in mutation breeding in rice in India, due to lack of proper standardization of doses. This experiment is an attempt to study the effect of proton beam on seedling growth parameters in rice.

Materials and Methods

Mature, healthy and uniform sized seeds of Indira BaraniDhan1(IBD-1) variety were selected. Prior to proton beam irradiation, seeds were subjected to viability testing and off-type seeds, if any, were removed.

Proton beam irradiation of the rice seeds was carried out using 14 MeV BARC-TIFR Pelletron accelerator facility at Tata Institute of Fundamental Research (TIFR), Mumbai. The actual proton beam energy selected from the accelerator was 14.52 MeV to account for the losses occurring in Gold target and Titanium window. Gold target of 1.35mg/cm^2 was used for the Rutherford scattering of ~ 2-3mm diameter beam from the Pelletron accelerator to a circular area of 11.35 cm^2 to ensure simultaneous irradiation of 50 grains, arranged in concentric layers in each plate (Fig.1). Titanium window (36mm diameter and 50 microns thick), at the exit of one meter long tube, formed a barrier between vacuum inside the tube and atmosphere on outer side. The beam profile was scanned using a PIN diode detector mounted on XY movement. The Proton flux is very high for detector even at a very low beam current. One milli meter collimator was mounted on the detector so as to efficaciously reduce the flux reaching the detector. For irradiation the detector setup was replaced by rice grains pasted in a specialized plastic cap. The flux incident on the grains was measured using another detector mounted at 30degrees in the scattering chamber. Multiple plastic caps were mounted on an irradiation wheel which can accommodate 66 samples which can be changed remotely without entering the irradiation area.

Various parameters relating to Proton beam penetration into the target were figured out using simulation program called SRIM (Stopping and Range of Ions in Matter) (Ziegler *et. al.*, 2010). Simulation inputs included seed density as 1.2g/cm^3 and incident energy as 14 MeV H^+. Bragg's peak for 14 MeV proton beam, appears at ~2.25mm. Using this simulation code, LET values at different depths of penetration, were estimated. It implied nearly constant LET values (min: 3.5keV/µm, max: 4.0keV/µm) at the entrance and the exit of ~600 µm embryonic region. To achieve a particular dose, required particle fluence was computed using the relationship given by Ziegler*et. al.* (2010):

Dose (Gy) = 1.6 X 10^{-9} × LET (keV/ µm) × particle fluence (protons/cm^2),

where higher LET value obtained in the embryonic region was used for the fluence estimation. The absorbed doses ranged from 0 to 500 Gy with a total of 15 doses *viz.,* 0 (control) 50, 100, 120, 140, 160, 180, 200, 220, 250, 280, 300, 350, 400 and 500 Gy. The dose uniformity was under ± 25 % in a 4 cm diameter culture plate.

Three replications, for each dose as well as for control, were used for irradiation and subsequent experimentation. After irradiation, ten seeds from each of the three replications / treatments were subjected to germination studies. Surface sterilization of seeds was carried out using 30% ethanol followed by rinsing in distilled water.

The sterilized seeds were kept immersed in distilled water and incubated overnight at 37 ˚C at 100 rpm in orbital shaker. Soaked seeds were then spread out on the sterilized blotting sheets placed inside different sterilized petri plates and subsequently, kept for germination in dark at room temperature in incubator. For all the treatments, data relating to germination was taken seven days after sowing. The emergence of coleoptiles and radical >1mm long was considered as germination (Lee *et.al.*, 1998). Germination percentage was calculated using the following formula:

$$\text{Germinationpercentage}= \frac{\text{Number of seeds germinated in 7 days}}{\text{Total number of seeds}} \text{ X } 100$$

For studying effect of proton beam on seedling growth parameters, Whatman filter paper no. 1 was cut into rectangular sheets of 15cm x 25cm dimension and the sheets were then folded, with each fold measuring 3cm in width. Ten small holes were made equidistantly in the trenches of the folded sheets and the latter were placed in germination racks. Seeds were placed near these holes with their embryonic portion facing the holes. The racks were positioned in rack stands with 3-4cm of water in it. The rack stands were kept in plant growth chamber (Sanyo, Japan) with a daily cycle of a 14h light/10h dark (Srivastava *et.al.*, 2014). For acquiring data on seedling growth parameters, seedlings were separated carefully and wiped properly, 15 days after the seeds were soaked. Data pertaining to plant growth such as shoot length, root length and total plant height was recorded on individual seedlings (Fig.2) and then averaged. Seedling vigor was calculated using formula proposed by Abdul-Baki and Anderson, 1973:

Vigor index = Germination x seedling length (shoot length + root length)

The experiment for lethal dose and growth reduction dose determination was done using completely randomized block design with three replications and each of three random blocks included 15 levels of proton beam treatment (including control). The significance of differences in averages, between irradiated (treatment) and non-irradiated (control), observed for various parameters, were investigated using Least significant difference (LSD) test at P-values less than 0.01. Analysis of variance was done using OPSTAT software (Sheoran *et.al.*, 1998). Probit analysis was carried out to determine the lethal dose 50 (LD50) of proton beam in rice (Finney, 1971 &1978).

Results and Discussion

This study was undertaken to standardize proton beam irradiation doses for IBD-1 variety of rice, using effect of irradiation on different growth parameters. Understanding the effect of proton beam on crop plants will help in its utilization for increasing crop diversity.

Lethal dose 50 (LD 50), the specific dose of radiation that kills 50% of the test material, is the optimum dose that causes high frequency of favourable mutations with minimum damage to the plant (Jency *et. al.*, 2016)). Determination of LD50 is crucial in order to reduce losses accrued to lethality caused by the physical mutagen and simultaneously retaining mutagenic effectiveness as it governs the availability of effective population size for the screening of a range of desirable mutants. Since LD50 value varies with biological materials, nature of mutagen and existing environmental conditions (Babaei *et. al.*, 2010), fixation of the value becomes inevitable prior to bulk mutagenesis of particular target. Doses lower than LD50 are favourable to the plant's recovery after treatment while the use of higher dose increases the probability to induce mutations either in positive or negative directions.

Germination percentage for increasing dose treatments showed linear decrease, significantly different over control (Table 2 & 4). For LD 50 determination using probit values, doses were converted into log scale (Table 1)and were plotted against probit of mortality percentage at different doses (Fig. 3). The plot of logarithmic values of doses used versus probit values of mortality percentage indicated 564.5Gy as the LD50 for IBD1.

Even at LD50, besides 50percent reduction in the survival of initial target population, retarded growth in the surviving fraction becomes a constraint in obtaining the expected number of mutants. The reduction in seedling growth after proton beam treatment has earlier been attributed to changes in ascorbic acid content and physiological injury and biochemical disturbances (Ussuf *et.al.*, 1974). It is, therefore, more useful to take into consideration growth reduction doses GR50 and GR30, which respectively signifies 50percent and percent reduction in growth parameter being considered, over respective control. The actual dose considered is heavily influenced by the objective of the experiment.

Growth reduction 50 (GR50) and Growth reduction 30 (GR 30) values were calculated for different phenotypic traits, evaluated after proton beam irradiation. The estimation of these values were based on the formulae for linear regression lines obtained in a plot of dose versus various growth parameters (shoot length, root length, total length of seedling, and vigor index) (Fig.4 & Fig. 5). Linear decrease in these different parameters of growth, measured for different dose treatments, showed significant differences over control (Table 2 & 4). The GR 50 values ranged from 253.53Gy (vigor index) – 393.01Gy (shoot length) and GR 30 values ranged from 152.47Gy (vigor index) – 256.50Gy (shoot length), for different growth parameters (Table 3). Since seed vigor reflects the sum total of all those properties of the seed which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence (Perry, 1978), GR 50 and GR 30 doses based on vigor index, would represent optimum doses in mutagenesis experiments.

The optimized doses will serve to initiate a large scale mutation breeding programme in rice variety IBD-1, without causing any significant alteration to the genotypic or phenotypic architecture of the crop plant.The identification of most effective mutagenic treatment and efficient mutagens is very essential to recover a high frequency and spectrum of useful mutations (Jency *et. al.*, 2016). The exact value of LD50, GR50, GR30 for proton beam varies with respect to differing radio-sensitivities of different rice varieties and also beam energy level.Owing to significance of seed vigor over the germination potential, as the latter does not reflect field performance of different genotypes under varied environmental conditions (Geetha*et. al.*, 2014), GR 50 and GR 30 attributes based on vigor index would serve as reliable parameters for extrapolating mutagenesis experiments to actual field conditions. This indicates proton beam dose between 152.47Gy (GR30) -253.53Gy (GR50) would be optimum dose range for irradiation of rice variety IBD-1. The optimum dose determination for potent physical mutagens like proton beam would aid in its utilization in rice improvement programs in future.

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References

Abdul-Baki, A.A. and Anderson, J.D. 1973.Vigor determination in soybean by multiple criteria.Crop Sci., **13**: p.630-633.

Babaei. A., Nematzadeh, G.A., Avagyan, V., Hamidreza, S.andPetrodi, H.2010. Radio sensitivity studies of the morpho-physiological characteristics in some Iranian rice varieties (*Oryzasativa* L.) in M1 generation. Afr. J. Agrl. Res.,**5**: p.2124-2130.

Bhat, N.N., Sapra, B.K. and Gupta, S.K. 2015.Proton Beam Set-up for Radiobiological Studies, BARC Newsletter, **346**: p. 5-7.

Challinor, A., Watson, J., Lobell, D., Howden, S., Smith, D. and Chhetri, N. 2014.A meta-analysis of crop yield under climate change and adaptation. Nat. Clim. Chang., **4(4)**: 287-291.

Finney, D.J. 1971. Probit analysis (3rd edition), Cambridge University press, Cambridge, UK.

Finney, D.J. 1978. Statistical method in biological assay (3rd edition), Charles Griffin and Co.

Geetha, V., Balamuruga, P. and Bhaskaran, M. 2014.Standardization of Vigor Test for Measuring the Vigor Status of Mustard Genotypes. Res. J. Seed Sci.,**7**: p.87-96.

Jency, J., Ravikesavan, R., Sumathi, P., &Raveendran, M. 2016. Determination of lethal dose and effect of physical mutagen on germination percentage and seedling parameters in kodomillet variety CO 3. Electron. J. Plant Breed., ***7*(4)**: 1122.

Lee, J., Lee, A. and Kwon, S. 2015. Effect of Proton Beam Irradiation on M1 Seeds and Seedling Growth in Rice. Plant Breed.Biotechnol., ***3*(4):** 384-388.

Lee, S.S., Kim, J.H., Hong, S.B. and Yun, S.H. 1998. Effect of humidification and hardening treatment on seed germination of rice. Korean J. Crop Sci., **43**: p. 157-160.

Magori, S., Tanaka, A. and Kawaguchi, M. 2010 Physically Induced Mutation: Ion Beam Mutagenesis. In: The Handbook of Plant Mutation Screening: Mining of natural and induced alleles, Khalid Meksem and Günter Kahl (Eds.), p.1-16.

Oladosu, Y., Rafii, M., Abdullah, N., Hussin, G., Ramli, A., Rahim H.et al. 2015. Principle and application of plant mutagenesis in crop improvement: a review. Biotechnol.Biotechnol. Equip., **30 (1)**: p.1-16.

Perry. D.A.1978. Report of the vigor test committee (1974–1977), Seed Sci. & Technol., 6: pp.159–181.

Rauf, S., Khan, A.A., TeixeriadaSilva, J.A. and Naveed, A. 2010.Consequences of Plant Breeding on genetic diversity. Int. J. Plant Breed.,**4**: p. 1-21.

Ray, D., Ramankutty, N., Mueller, N., West, P. and Foley, J. 2012. Recent patterns of crop yield growth and stagnation. Nat. Commun., **3(1)**.

Sheoran, O.P., Tonk, D.S., Kaushik, L.S., Hasija, R.C. and Pannu, R.S. 1998. Statistical Software Package for Agricultural Research Workers. In: Recent Advances in information theory, Statistics & Computer Applications, D.S. Hooda and R.C. Hasija (Eds.), Department of Mathematics Statistics, CCS HAU, Hisar, p. 139-143.

Srivastava, A., Srivastava, S., Mishra, S., D'Souza, S. and Suprasanna, P. 2014. Identification of redox-regulated components of arsenate (AsV) tolerance through thiourea supplementation in rice. Metallomics, **6(9)**: 1718-1730.

Tanaka, A., Shikazono, N. and Hase, Y. 2010. Studies on Biological Effects of Ion Beams on Lethality, Molecular Nature of Mutation, Mutation Rate, and Spectrum of Mutation Phenotype for Mutation Breeding in Higher Plants. J. Radiat. Res., **51(3):** 223-233.

Ussuf, K.K., & Nair, P.M. 1974. Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes.Radiat. Bot., **14(4)**: 251-256.

Ziegler, J., Ziegler, M., &Biersack, J. 2010. SRIM – The stopping and range of ions in matter. Nucl. Instr. Meth. Phys. Res.B: Beam Interactions with Materials and Atoms, **268(11-12)**: 1818-1823.

Table 1. Dosage effect of proton beam irradiation on mortality (expressed as percentage relative to control) and probit values of rice variety IBD1.

Dose (Gy)	Log (dose)	% Mortality	Probit of % mortality
0	—	—	—
50	1.699	16.67	4.01
100	2	4.17	3.25
120	2.079	8.33	3.59
140	2.146	12.5	3.82
160	2.204	8.33	3.59
180	2.255	16.67	4.01
200	2.301	33.33	4.56
220	2.342	20.83	4.16
250	2.398	16.67	4.01
280	2.447	25	4.33
300	2.477	33.33	4.56
350	2.544	45.83	4.87
400	2.602	50	5
500	2.699	62.5	5.31

Table 2. Effect of proton beam on germination and growth parameters of seedlings of IBD1 variety of rice

Dose (Gy)	% Germination	Shoot length (cm)	Root length (cm)	Plant length (cm)	Vigor index
0	100	12.22	15.5	27.72	2772.22
50	83.33	12.59	13.93	26.52	2211.28
100	95.83	10.92	14.51	25.43	2440.6
120	91.67	12.6	14.13	26.73	2457.85
140	87.5	12.46	13.19	25.65	2244.31
160	91.67	10.62	12.74	23.37	2162.5
180	83.33	10.92	11.87	22.78	1891.19
200	66.67	8.73	10.17	18.9	1268.61
220	79.17	9.93	11.43	21.36	1687.33
250	83.33	6.29	6.63	12.93	1067.08
280	75	6.52	7.46	13.97	1048.1
300	66.67	6.25	7.61	13.86	906.24
350	54.17	7.33	5.41	12.74	673.89
400	50	6.28	6.25	12.52	626.08
500	37.5	5.39	4.54	9.93	372.5
Overall mean	76.39	9.27	10.36	19.63	1588.65
C.D.	12.614	1.949	2.12	3.561	390.315
SE(m)	4.332	0.669	0.728	1.223	134.044
SE(d)	6.126	0.947	1.03	1.73	189.567
C.V.	4.823	2.508	4.174	4.793	3.614

Table 3. Evaluation of GR50 and GR30 for Shoot length, Root length, Plant height and Vigor index, based on 50% and 30% reductions in their respective values over the control.

Growth parameter	Control values	50% reduction value	30% reduction value	GR 50 (Gy)	GR 30 (Gy)
Shoot length	12.21cm	6.109 cm	8.55 cm	393.01	256.5
Root length	15.50 cm	7.752 cm	10.85 cm	317	197.73
Plant height	27.722 cm	13.86 cm	19.40 cm	347.99	221.69
Vigor index	2772.22	1386.11	1940.55	253.53	152.47

Table 4. ANOVA test for evaluating significance of effect of proton beam irradiation on different growth parameters considered.

Source	DF	Mean Sum of Squares				
		% Germination	Shoot length (cm)	Root length (cm)	Plant length (cm)	Vigor index
Treatment	14	979.66*	21.92*	41.34*	120.62*	1822495.97*
Error	28	56.3	1.34	1.59	4.49	53903.7

* F-value calculated implied highly significant differences (between control and the treatment) for all the traits considered at $p<0.01$

Fig. 1. IBD1 variety seeds arranged on plastic plates (4cm diameter), with their germ facing centre of the plate, for irradiation.

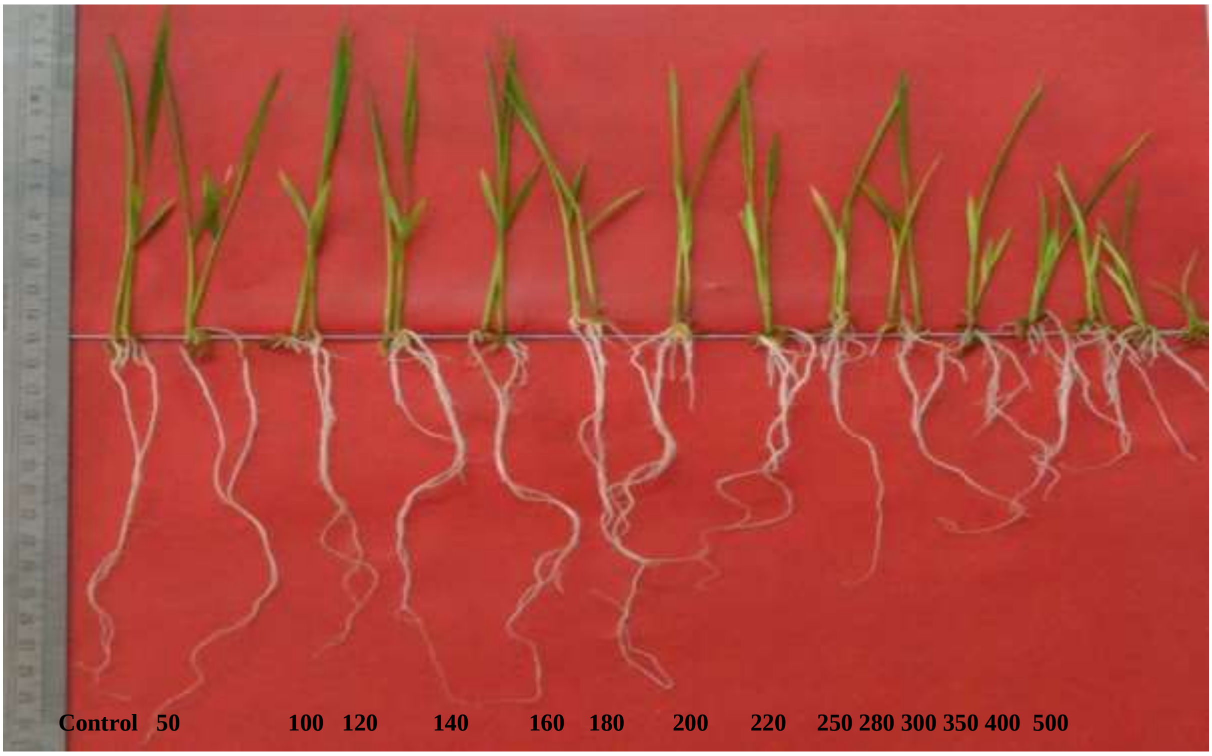

Fig. 2. Effect of different doses of proton beam on seedling growth parameters (control represents untreated sample, treatment dose values measured in Gy).

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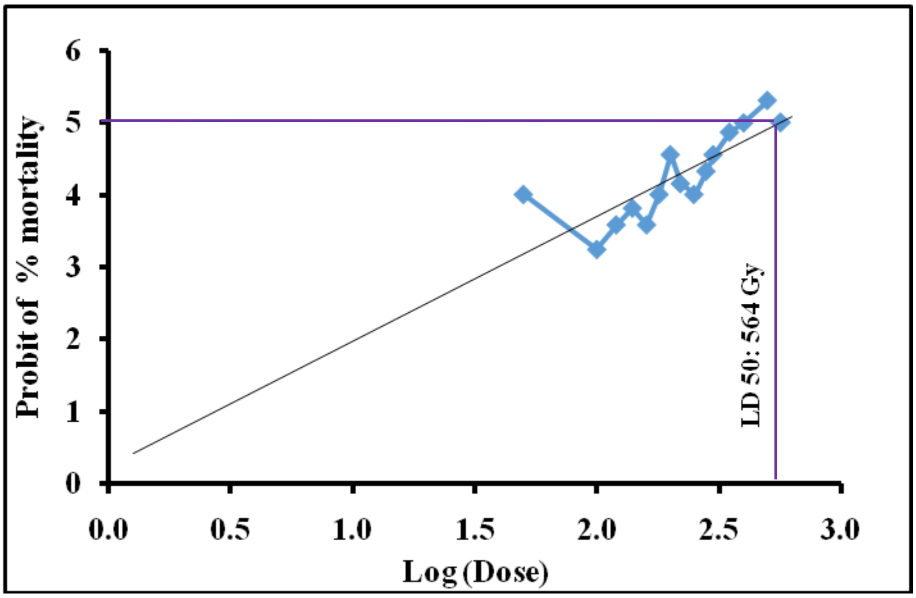

Fig. 3. LD 50 determination from a plot between probit of % mortality caused and logarithmic values of dose (Gy), for proton beam irradiation of IBD1 variety.

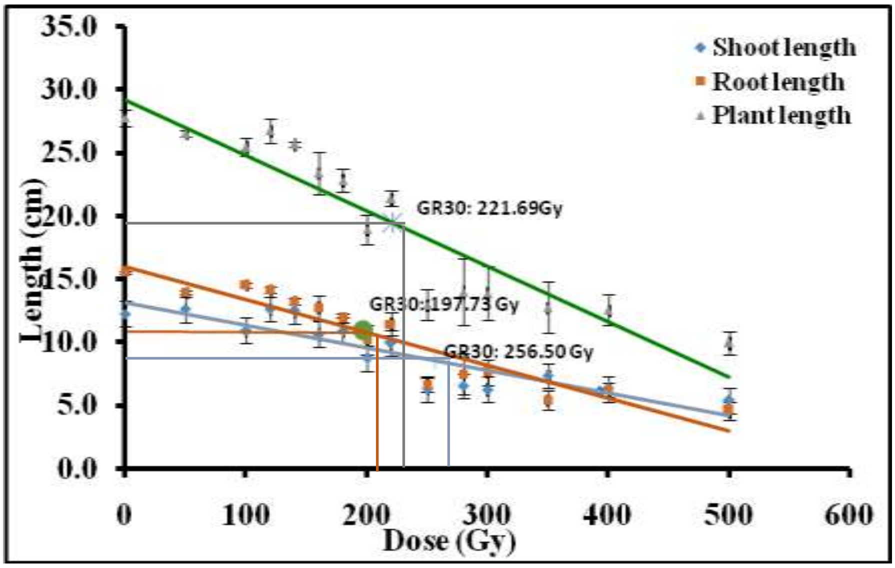

Fig. 4. GR50 and GR 30 determination for Shoot length, Root length, Plant length (However, values for GR30 only has been indicated in the graph. For GR50, refer to table 4).

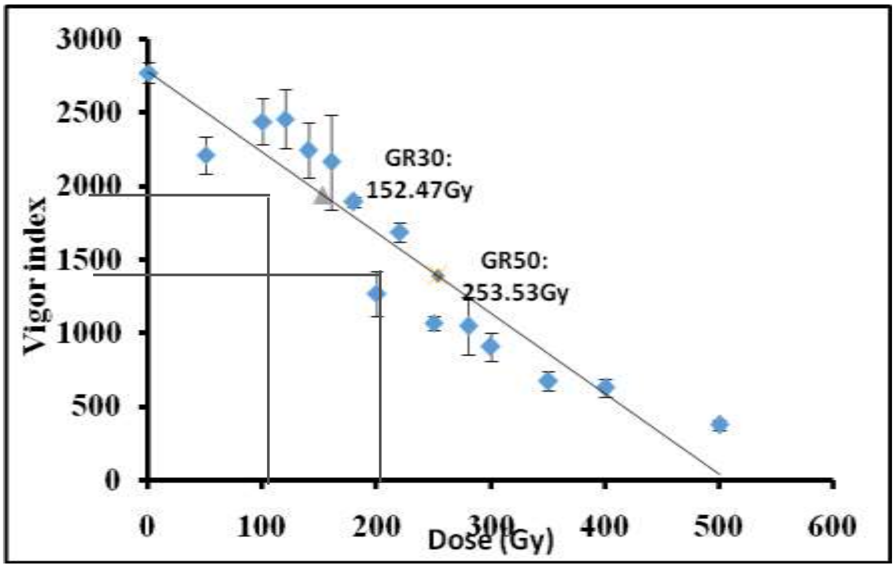

Fig. 5. GR50 and GR 30 determination for Vigor index

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