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Short Communication

Standardization of GR_{50} dose of gamma rays for mutation breeding experiments in safflower (*Carthamus tinctorious* L.)

Rajeev Shrivastava*, Suvendu Mondal[1], Nirmala B. Patel, Shampa Purkayastha and Yengkhom Linthoingambi Devi

Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur, Chhattisgarh 492 012; [1]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085

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Abstract

GR_{50} dose of gamma rays was optimized in four different genotypes of safflower. *In vitro* experiment during seedling stage detected very high GR_{50} value of 579.2 Gy based on reduction of root length. Field experiments on four genotypes revealed significant difference in GR_{50} values among safflower genotypes. The GR_{50} values varied in between 248.7 to 374.3 Gy based on the seedling height data at 42 days after sowing. RVS 2012-13 was most sensitive to radiation, while Annegiri 1 was the tolerant.An optimal dose of 300 Gy was found suitable for large scale mutation breeding experiments in safflower.

Key words: Gamma rays, GR50 dose, Mutation breeding, Mutation frequency, Safflower

Induced mutagenesis is an important breeding technique to create genetic variability in crop species. Use of suitable mutagens and handling of mutant generations are very important steps towards the isolation of superior mutants in plant. Safflower (*Carthamus tinctorius* L.) is an ancient oilseed crop valued for its quality oil and dye from its petals. It has very narrow genetic variability for morphological, agronomical and biochemical traits (Rampure et al. 2014). Few reports are available towards the use of mutation technique in improvement of safflower (Mallikarjunradhaya 1978; Ramchandram and Goud 1983; Velasco et al. 2000; Kotcha et al. 2007). But most of them were depicted the usage of chemical mutagenesis. Usage of gamma rays and other physical mutagens are limited in the literature, however, there are several reports available in literature about the successful and beneficial use of gamma rays in crop improvement (Kundu and Dubey 2020; Mahla et al. 2018; Pallavi and Badere 2020). Limited studies have documented mutagenic potential of gamma rays on somatic and gametic cells of safflower (Kumar and Srivastava 2010; Verma and Shrivastava 2014; Rampure et al. 2017). The median lethal dose (LD_{50}) and the median growth reduction (GR_{50}) are parameters utilized to establish the adequate gamma irradiation dose for mutations induction. Notably, both LD_{50} and GR_{50} parameters 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 (Álvarez-Holguín et al. 2019). Therefore, the first step in a mutagenesis-based breeding process is to determine LD_{50} and GR_{50}values. Reports on GR_{50} dose (gamma rays) on safflower are not mentioned correctly in literature. In the manual of mutation breeding (FAO/ IAEA 2018), very high GR_{50} dose is prescribed for safflower. Such high GR_{50} dose limits the stand of M_1 plant and also brings down M_2 population size. For successful mutation induction in plants, the applied dose should be below GR_{50} so that the size of M2 population in field and higher mutation efficiency can easily be obtained. Present research effort intended to standardize GR_{50} dose of gamma rays towards

*Corresponding author's e-mail: rajeevigkv@gmail.com
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isolation of mutants in safflower. Further, the study also revealed the varietal difference in radiation sensitivity among safflower genotypes.

Pure seeds of RSS 7 genotype were irradiated with 100 to 800 Gy of gamma rays in a ^{60}Co Gamma Cell 5000 irradiator (BRIT, Mumbai, India) at a dose rate of 40.9 Gy min^{-1}.The germinated healthy seedlings were transferred to Gibson tube containing 0.5 x Steinbergs solution for seedling growth at 24°C, 65% relative humidity, 12 h light and 12 h dark. After 15 days, seedling height and root length were measured. For *in vivo* experiments, seeds from four different genotypes, RVS 2012-13, RSS 3, RSS 7, and Annegiri 1 were exposed to 100 to 800 Gy gamma radiation as per above method. Each genotype was sown in a block and different doses were kept in different rows in each block along with control in *rabi-summer* 2016-17. Since safflower has rosette growth habit for 21- 30 DAS, the seedling height was taken at 42 and 72 DAS in the field. For each dose, seedling height (*in vivo*) or shoot/root length (*in vitro*) data were obtained from 10 seedlings. These shoot/root length data were then transformed into 'percentage reduction in length over control' and percentage reduction data was converted to probit values and regression analysis was made against the log value of doses. Dose at 50% growth reduction (GR_{50}) values were calculated at 95% confidence limit. The GR_{50} data of four genotypes from *in vivo* experiments were used to reveal significant genotypic difference based on analysis of variance using IRRISTAT 2.0 (IRRI, 2003). For the actual mutation breeding experiments, two lots (each contain 1000 seeds) of seeds from each genotype were irradiated with 500 Gy and 300 Gy as mentioned in above. Both the lot was grown separately in different blocks during *rabi-summer* 2016-17. The seeds were harvested from main capitulum of each M_1 plant and kept in a single packet. The M_2 seeds were grown as plant to row progenies during *rabi-summer* 2017-18 at experimental field facility, IGKV, Raipur. Mutants were identified and tagged and tested for their true breeding behavior by growing plant to row progenies in the M_3.

Absorbed dose of ionizing radiation plays a trade off between radiation-induced damage/lethality and effective number of mutants in a mutation breeding experiment. Thus, dose optimization in case of physical mutagenesis is the prime step in mutation breeding (Ahloowalia et al. 2004). Doses of mutagen that lead to 50% lethality or 50% growth reduction are considered as LD_{50} or GR_{50}, respectively (Viana et al.2019). Whereas, the dose of a mutagen that achieves the optimum mutation frequency with the least possible unintended damage is considered as the optimal dose (Mba et al. 2010). This optimal dose lies in between GR_{30} and GR_{50} values (Roy 2019). In the present study, *in vitro* experiment revealed dose dependent gradual shoot and root length reduction in safflower seedlings. Less growth reduction in shoot was observed compared to root due to rosette habit. The calculated GR_{50} was 579.2 Gy (based on root length reduction) and 764 Gy (in terms of shoot growth).This calculated GR50 dose is well matched with the published report where the dose varied from 600 to 700 Gy (FAO/IAEA 2018). Thus, we initially selected an optimal dose of 500 Gy for mutation induction. But more lethality (90%) was noticed in actual field condition. Such observation forced us to conduct field-based radiation sensitivity experiments (*in vivo* experiment) for safflower. In radiation sensitivity experiments, seedling height was considered a prime criterion to assess GR_{50} dose (Kodym et al. 2011). *In vivo* experiments revealed no emergence of seedling at 700 Gy and above in all the four tested genotypes. Safflower has unique rosette behaviour in seedling stage that varies from 20 to 35 DAS (Singh and Nimbkar 2006). Due to this rosette habit, seedling height data were collected at 42 DAS and 72 DAS. Gradual seedling height reduction was observed for all the tested genotypes in response to the incremental dose. ANOVA revealed a significant difference in GR_{50} dose among genotypes in both the phenophase of the crop (42 DAS & 72 DAS) (Table 1).

Table 1. Analysis of variance of GR50 dose over four genotypes tested in this study

Source of variation	Degree of freedom	Mean sum of squares of GR50 dose		F calculated		F table (P = 0.01)
		DAS72	DAS 42	DAS72	DAS 42	
Replication	9	55.17	1518.4	0.27	5.35	3.15
Genotype	3	37339.51**	9964.8**	183.67	35.10	4.61
Error	27	203.29	283.86			

GR_{50} value for RVS2012-13 was the least: 248.7 Gy (42 DAS) and 301.2 Gy (72 DAS). Annegiri 1 had the highest GR_{50} value: 374.3 Gy (42 DAS) and 447.6 Gy (72 DAS). The second least sensitive genotype was RSS 7: 257.9 (42 DAS) and 477.3 Gy (72 DAS). The

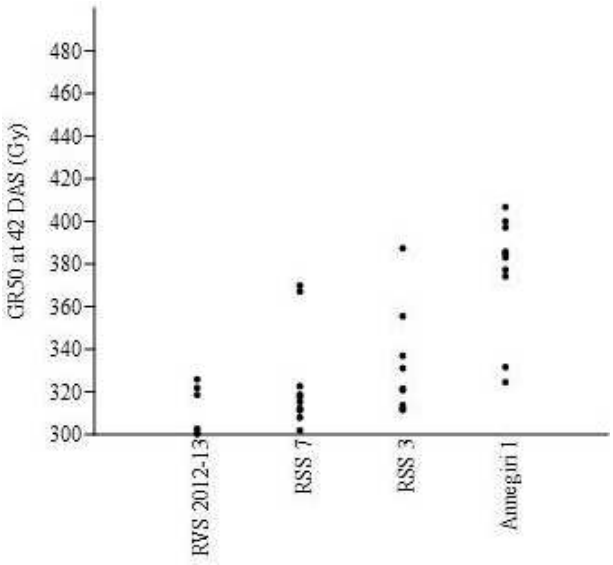

Fig. 1. Jitter plot of GR50 doses of four safflower genotypes at 42 DAS

other moderate sensitive genotype was RSS 3: 294.8 Gy (42 DAS) and 427 Gy (72 DAS). There is a significant difference in GR50 values among the genotypes at 42 DAS (Fig. 1) while GR_{50} values of three genotypes (RSS 3, RSS 7 and Annegiri 1) did not differ significantly at 72 DAS and thus consider same in terms of their radio-sensitivity.This variation in GR_{50} dose among genotypes signified the genotypic difference for radiation sensitivity. Such a significant difference in radiation sensitivity was noted in case of electron beam implanted rice genotypes (Shu et al. 1996), rice landraces irradiated with various physical mutagen (Sao et al. 2020) and groundnut genotypes exposed to gamma rays and electron beam (Mondal et al. 2017). Based on the derived GR_{50} values in *in vivo* experiment, a dose of 300 Gy was applied to 1000 seeds of each genotype to raise M1 population in field. The field emergence of M1 plant at this dose was almost 75% and found to be sub-lethal. Shorting of internodes, chlorosis, mosaic, and other leaf deformation were noticed in M1 plants. The least plant population was obtained in case of RVS 2012-13 (more sensitive to gamma rays) and the highest in Annegiri 1 (less sensitive to gamma rays). From the above M1 progenies large numbers of M2 population were grown in field. In total, 92000 plants were obtained in whole M2 population of four genotypes. Variants like early rosette, early flowering, capitulum size, plant height, leaf shape, bud shape, appressed branches and fused capitulum etc. were tagged in M2 generation. In total, 250 plant progenies were grown in the M3 to test true breeding behavior of each variant identified in the M2. Of them, 67 true breeding mutants were obtained from this large-scale mutation breeding experiments. The overall mutation frequency obtained in this study was approximately 7.3×10^{-4}.

The present experiment has demonstrated the usage of proper calibrated dose for induction of large number of mutants in safflower. The study reiterates the proper calibration of GR_{50} dose before conducting any mutation breeding experiment and generation of large M2 population for the isolation of economical mutants.

Authors' contribution

Conceptualization of research (RS, SM); Designing of the experiments (RS, SM, NBP); Contribution of experimental materials (RS, SM); Execution of field/ lab experiments and data collection (RS, NBS, SP, YLD); Analysis of data and interpretation (RS, NBP, SP, YLD); Preparation of manuscript (RS, SM).

Declaration

The authors declare no conflict of interest.

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