



Co-60 gamma irradiation induces variation in F₃ watermelon resistance to *Macrophomina phaseolina*

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Abstract

Gamma radiation has been employed to induce mutations in seeds and to develop disease-tolerant cultivars. This study aimed to assess resistance to *Macrophomina phaseolina* in F₃ watermelon (*Citrullus lanatus*) genotypes derived from seeds of the cultivar Sugar Baby irradiated with 200 Gy of Co-60 gamma rays. The experiment was conducted in a greenhouse using a completely randomised design, with 14 treatments, and five replicates. The assay was repeated. The materials evaluated included the melon (*Cucumis melo*) accession Ag-15591Ghana (G1), non-irradiated Sugar Baby watermelon (G2), and five irradiated watermelon genotypes (G3–G7), obtained through controlled manual pollinations from seeds previously exposed to 200 Gy of gamma radiation. Plants were inoculated with *M. phaseolina* isolate A11P17, and disease severity was assessed 30 days post-inoculation using a 0–5 rating scale. Genotypes G1 and G2 were susceptible, whereas the irradiated genotypes, particularly G4, exhibited higher frequencies of resistant individuals. The observed genetic variability suggests that gamma irradiation is a promising strategy for improving resistance to *M. phaseolina* in watermelon, with the potential to reduce disease impact and support yield stability.

Keywords Charcoal rot · *Citrullus lanatus* · Genetic variability · Resistance breeding

Watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) is a widely cultivated cucurbit, particularly in tropical and subtropical regions (Bantis and Koukounaras 2023). In Brazil, the Northeast offers favourable conditions for its cultivation (Silva et al. 2021). Among the cultivated varieties, ‘Sugar Baby’ is valued for its sweet flavour and fruit quality (Pp et al. 2022).

In recent years, root diseases caused by soilborne pathogens have become a major concern in Brazil, with *Macrophomina phaseolina* emerging as the predominant species isolated from collapsed watermelon roots in commercial growing areas (Sales Júnior et al. 2019; Pisani et al. 2021;

Wu et al. 2022). The pathogen persists in soil as long-lived microsclerotia that germinate under favourable conditions to infect roots, and its wide host range, exceeding 500 plant species, favours rapid reinfestation (Javaid et al. 2017; Javed et al. 2021). Managing charcoal rot is challenging, and no complete resistance has been reported in cultivated cucurbit species (Basandrai et al. 2021; Melo et al. 2021; Alves et al. 2025).

Gamma radiation has been applied to induce mutations that enhance disease resistance in crops (Bhoi et al. 2022). Low doses have shown effectiveness against pathogens such as *Fusarium* spp., *Rhizoctonia solani*, and *M. phaseolina*, while also promoting seed germination and plant growth (Ikram et al. 2010; Jaipo et al. 2019). Alterations in physiological and biochemical processes in plants have been used to investigate their interactions with pathogens (Gajbar et al. 2021; Saadati et al. 2022; Zhang et al. 2023).

This study aimed to assess whether gamma irradiation at 200 Gy could induce resistance to *M. phaseolina* in F₃ Sugar Baby watermelon genotypes, by evaluating disease severity following artificial inoculation.

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Fig. 1 Charcoal rot symptoms caused by *Macrophomina phaseolina* isolate A11P17 at 30 days after inoculation (DAI). **(a)** Non-inoculated, non-irradiated watermelon control. **(b)** Inoculated, non-irradiated melon accession (G1). **(c)** Inoculated, non-irradiated watermelon genotype (G2). **(d)** Inoculated, irradiated watermelon genotype (G4). **(e)** *M. phaseolina* colony on BDAS medium obtained from infected stem fragments

Table 1 Disease severity, relative treatment effect (RTE), reaction class of melon and watermelon genotypes to *Macrophomina phaseolina*

Genotype	Severity $\pm$ SD	RTE	LL	UL	Reaction
G1	3.2 $\pm$ 0.79	0.77 ^a	0.67	0.84	SU
G2	2.7 $\pm$ 1.34	0.65 ^{ab}	0.50	0.77	SU
G3	2.0 $\pm$ 1.16	0.52 ^{bc}	0.38	0.66	MR
G4	0.8 $\pm$ 0.63	0.24 ^d	0.17	0.34	AR
G5	1.8 $\pm$ 1.32	0.45 ^{bcd}	0.31	0.61	MR
G6	1.4 $\pm$ 1.26	0.37 ^{cd}	0.24	0.54	MR
G7	1.9 $\pm$ 1.45	0.49 ^d	0.32	0.66	MR

F (df1 = 5.14; df2 = 52.48) = 5.02 ($p = 0.0007$)

SD: standard deviation. Disease reaction classes: SU=susceptible; MR=moderately resistant; HR=highly resistant. RTE values followed by the same lowercase letter do not differ significantly according to Noguchi et al. (2020) ($p > 0.05$)

Five F₃ genotypic groups (G3–G7) of Sugar Baby watermelon were evaluated, derived from seeds irradiated with 200 Gy of Co-60 gamma rays using a Gammacell-220 source at the Centro de Energia Nuclear na Agricultura, Universidade de São Paulo (CENA, USP, Brazil). These genotypes originated from manual self-pollination in the F₂ generation. Commercial Sugar Baby seeds (G2) were used as a susceptibility control, and the melon accession Ag-15591Ghana (G1) as a reference for partial resistance to *M. phaseolina* isolates (Ambrósio et al. 2015; Linhares et al. 2020).

Seeds were disinfected in 2.5% sodium hypochlorite and sown in 200-cell trays. At 14 days after sowing (DAS), seedlings were transplanted into 1 L pots filled with a 2:1 (v/v) mixture of sterilised soil and Tropstrato HT[®] substrate. Plants were irrigated daily.

The *M. phaseolina* isolate A11P17 was obtained from diseased watermelon plants in Rio Grande do Norte, Brazil. Inoculation followed the toothpick method (Ambrósio et al. 2015; Negreiros et al. 2019). Sterilised toothpicks were placed in Petri dishes containing potato dextrose agar supplemented with tetracycline sulfate (PDAS), inoculated

with fungal plugs, and incubated at 28 $\pm$ 2 °C in the dark for seven days.

At 15 DAS, seedlings were inoculated by inserting colonised toothpicks into the hypocotyls, ~1 cm above the soil. Control plants received sterile toothpicks. All plants were maintained under greenhouse conditions (average temperature of 35 $\pm$ 2 °C).

Disease severity was assessed 30 days post-inoculation (DPI) using a modified 0–5 rating scale adapted from Ambrósio et al. (2015): 0=asymptomatic; 1=<3% affected; 2=3–10%; 3=11–25%; 4=26–50%; 5=>50% affected. Based on mean severity, genotypes were classified as immune (0), highly resistant (0.1–1.0), moderately resistant (1.1–2.0), susceptible (2.1–4.0), or highly susceptible (4.1–5.0) (Salari et al. 2012).

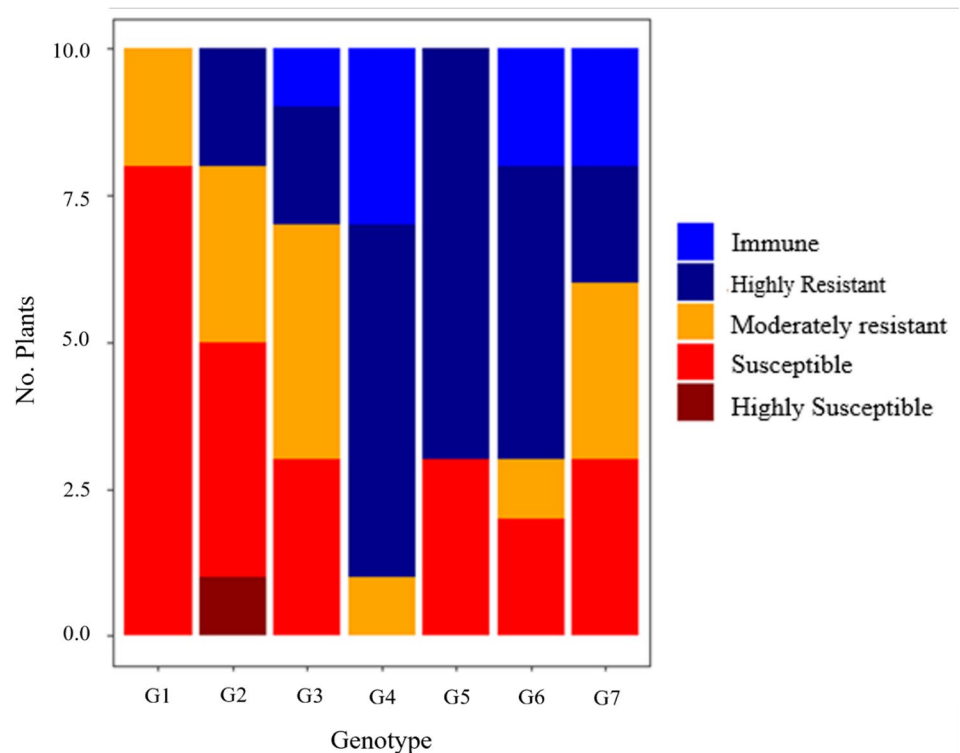
The experiment followed a completely randomised design with 14 treatments, and five replicates. The trial was repeated for validation. Nonparametric ANOVA-type statistics (Brunner et al. 2017) and Relative Treatment Effect (RTE) values (Konietschke et al. 2010) were used to assess genotype effects. Multiple comparisons followed Noguchi et al. (2020). Analyses were performed in R (R Core Team 2023).

The *M. phaseolina* A11P17 isolate induced typical charcoal rot symptoms, confirming its pathogenicity (Fig. 1). Pathogenicity was further confirmed by fulfilling Koch's postulates, through reisolation of the pathogen from symptomatic plants and its absence in control plants.

Statistical analysis revealed significant differences among genotypes ($p = 0.0007$). The highest RTE was recorded for G1 (0.77), followed by G2 (0.65), indicating high susceptibility in the non-irradiated genotypes. In contrast, all irradiated genotypes (G3–G7) had reduced RTE values, with G4 showing the lowest (0.24), suggesting a more resistant profile (Table 1).

Mean disease severity scores indicated that G1 and G2 were classified as susceptible. Irradiated genotypes

Fig. 2 Distribution of plants across resistance categories to *Macrophomina phaseolina*, assessed in seven genotypes. G1: non-irradiated melon accession Ag-15591Ghana; G2: non-irradiated watermelon cultivar ‘Sugar Baby’; G3–G7: F₃ genotypes of ‘Sugar Baby’ derived from seeds treated with 200 Gy of gamma radiation



exhibited moderate (G3, G5, G6, G7) to high resistance (G4) and reaction class distribution further confirmed these patterns (Fig. 2). While most individuals in G1 and G2 were susceptible, the irradiated lines had more immune or resistant plants, particularly in G4, where all individuals were classified as resistant. This suggests the presence of favourable alleles in this segregating population.

The observed variation may be attributed to specific host–pathogen interactions and the high pathogenic variability of *M. phaseolina* (Jyothi et al. 2020), which could explain the susceptibility of both G1 and G2 to this isolate. The results align with previous studies highlighting induced mutagenesis as a strategy to enhance disease resistance (Zulkifli et al. 2024). Molecular analyses of gerberas (*Gerbera jamesonii*) moderately resistant to powdery mildew have shown that gamma radiation acts directly at the genetic level (Ghani and Sharma 2019). Thus, the G4 genotype, characterised by a high frequency of resistant individuals, may represent a group capable of withstanding high pathogen pressure.

The application of gamma radiation (Cobalt-60) to seeds of susceptible maize genotypes induced significant genetic variability, particularly associated with resistance to maize streak disease (Afram et al. 2024). Similarly, irradiated soybean genotypes showed increased phenolic content linked to tolerance against *M. phaseolina* (Samudio-Oggero et al. 2025). Even within a single cultivar, genotypic responses to irradiation can vary due to genetic heterogeneity, seed positioning in the irradiation chamber, and intrinsic differences

in DNA repair and antioxidant mechanisms (Khare et al. 2025), factors that may account for the variation in resistance levels among irradiated watermelon genotypes.

Although complete resistance, characterised by the exclusive presence of immune individuals among all irradiated genotypes, was not observed, the reduction in disease severity and increased survival associated with highly resistant (HR) watermelon plants may have economic relevance, as they help maintain yield potential under disease pressure (Nelson et al. 2021). Evidence from other crops supports this trend. In irradiated pepper (*Capsicum annuum*), resistant individuals selected in the M₃ generation produced healthy fruits and led to the development of stable M₄ lines resistant to anthracnose (Puripunyanich et al. 2024). Taken together, these findings underscore the importance of using homozygous material in future studies. Nobre et al. (2025) demonstrated that pure soybean lines, when irradiated, allowed clearer detection of induced variability, reinforcing gamma rays as an effective tool in resistance breeding.

Gamma irradiation at 200 Gy induced genetic variability associated with resistance to *Macrophomina phaseolina* in F₃ watermelon genotypes. While non-irradiated genotypes were susceptible, irradiated lines, especially G4, showed reduced disease severity and a higher frequency of resistant individuals. These results support the use of induced mutagenesis in developing resistant germplasm and highlight its potential in cucurbit breeding programmes targeting charcoal rot.

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Data availability Not applicable.

Declarations

Ethical approval Not applicable.

Conflict of interest The authors declare no conflicts of interest.

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